Bone Targeted Radiofrequency Ablation (RFA) Electrodes for the Treatment of Appendicular and Vertebral Metastases

by
Padina Pezeshki

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Institute of Biomaterials and Biomedical Engineering
University of Toronto

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Abstract

Bone metastases, an unfortunate and frequent occurrence in cancer, can result in skeletal related events, including pain, pathologic fractures, and hypercalcemia. Treatment strategies, such as surgery and/or radiation therapy, can be limited by invasiveness, maximum dose levels, and radio-resistivity. In this context, image-guided minimally invasive thermal treatment modalities such as radiofrequency ablation (RFA) have gained interest in the treatment of bone metastases. RFA conducts an alternating current through a probe placed within the tumour, resulting in ionic excitation of cells and frictional heating. RFA is reliant upon thermal and conductive properties of the tissue and leads to coagulative necrosis. Current technology developed and tested in soft tissues is limited by carbonization, small and unpredictable zones of ablation, particularly when used in bone. The lower conductive properties of bone tissue and proximity of bone to critical structures further challenge RFA application. This thesis focuses on design and evaluation of two novel bone-targeted RFA electrodes, a bipolar cooled RF (BCRF) and a solenoid-shaped (Bone Coil) RF probe, to improve the size and efficacy of RFA in bone. RFA was evaluated using healthy porcine and diseased lapine models with outcomes assessed through Magnetic
Resonance Imaging (MRI) and histologic analysis of bone and tumour tissue. A cadaveric model was used to evaluate the role of RFA on spinal stability alone and in combination with vertebroplasty. Both BCRF and Bone Coil RF ablation were safe and effective in the spine. T2-weighted and contrast-enhanced T1-weighted MRI sequences two weeks post treatment were found to be most effective for image-based therapeutic evaluation. BCRF ablation yielded an eight-fold reduction in tumour volume in the rabbit femur. Treatment necrotized osteoblasts and osteoclasts comprehensively, whereas osteocytes were found to be more resilient to RFA. New bone formation and remodelling was observed at the ablation zone periphery. RFA alone led to reduced vertebral stability, but a restoration of strength and stability comparable to healthy levels was achieved when RFA treatment was combined with cement injection localized into the posterior portion of the vertebral body. Overall, this work motivates the future use of bone-targeted RF technology in the treatment of skeletal metastases.
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Chapter 1
Introduction

1.1 Review of Bone Metastasis

Bone metastasis is a common occurrence that presents in over 70,000 Canadians annually (1), or in over 85% of cancer patients at the time of death (2). Many primary cancers spread to the skeleton with a postmortem incidence of about 70% in breast and prostate carcinomas and 40% in thyroid, kidney, and lung cancer patients (3, 4). Table 1 demonstrates the incidence of skeletal metastases based on autopsy and scintigraphic examinations.

A number of factors are responsible for development of metastatic bone disease. The original "seed and soil" hypothesis put forth by Paget in 1889, still holds valid. According to this theory, primary cancer cells (seeds) preferentially colonize in organs, such as the skeleton, where the microenvironment (soil) is fertile (5, 6). Physical properties of bone such as hypoxia, acid pH, and a high extracellular calcium concentration have been shown to promote tumour growth (5, 7).

The mechanistic theory, which states that tumour cells will metastasize in locations anatomically close to their primary site, provides further rationale as to the high incidence of skeletal metastases. Tumours may spread to the spine through hematogenous spread (most common), direct extension (as in Pancoast's superior sulcus tumour), or occasionally through neuroforamina (as in lymphoma) (8).

The spine is the most common site of bone metastases, representing more than 90% of the tumours of the skeleton (9), followed by the pelvis, ribs, femur, humerus and skull (10). Each year about 5% of cancer patients or approximately 61,000 persons in the US develop bone metastases in their spines (11). Table 2 demonstrates the distribution of skeletal metastases based on some of the common carcinomas. Two thirds of spinal metastases occur in the thoracic vertebrae and the remainder occur twice as frequently in the lumbar spine as compared to the cervical region (12). Generally, the posterior portion of the vertebral body is first affected and gradually the anterior body, lamina and pedicles also become involved (8, 13). Due to modern advanced diagnostic tools, particularly MRI, spinal lesions are now detected at a much earlier stage, when their size may be smaller. These lesions often lie near critical neural and vascular...
structures (9). The anatomical areas typically affected by bone metastasis that are critical to functional mobility and ambulation are the spine (i.e. vertebral lesions), and proximal weight bearing long bones (i.e. femoral lesions). This has important implications (see section 1.5.6, pg 28) when considering the development of therapies (such as bone-targeted radiofrequency) to treat what are typically larger structural lesions in human bones.

The duration of survival from the time of diagnosis of bone metastasis varies from years (for prostate and breast cancer patients) to months (for advanced lung cancer patients) (4). During this time, skeletal metastases may cause considerable morbidity, namely: pain, hypercalcemia, pathological fracture, spinal cord and nerve compression (in 5% of patients (8)), as well as bone marrow infiltration or aplasia. Collectively, these sequelae are referred to as “skeletal-related events” (SRE) (6, 7, 14). A patient suffering from bone metastases generally experiences a SRE every 3-6 months (5).

Bone metastases can be classified as osteolytic, osteoblastic or a combination of the two (mixed lesions). Patients may present with different lesion types at distinct skeletal sites. Initial presentation with osteolytic metastases is considerably more common (95%) and are characterized by increased osteoclastic activity and bone resorption (6, 8). Osteoblastic metastases are characterized by excessive production of osseous tissue due to activated osteoblasts. Osteoblastic lesions result most commonly from prostate carcinomas (8). Figure 1 shows a histological comparison of normal bone versus osteolytic and osteoblastic metastases.

Table 1. Incidence of skeletal metastases, based on autopsy and scintigraphic examinations. Reproduced with permission from Galasko, 1981(15). Copyright Wolters Kluwer journals.

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary carcinoma</td>
<td>47-85</td>
</tr>
<tr>
<td>Prostatic carcinoma</td>
<td>33-85</td>
</tr>
<tr>
<td>Thyroid carcinoma</td>
<td>28-60</td>
</tr>
<tr>
<td>Renal carcinoma</td>
<td>33-40</td>
</tr>
<tr>
<td>Bronchial carcinoma</td>
<td>30-60</td>
</tr>
<tr>
<td>Uterine cervical carcinoma</td>
<td>50</td>
</tr>
<tr>
<td>Vesical carcinoma</td>
<td>42</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>9</td>
</tr>
<tr>
<td>Rectal carcinoma</td>
<td>8-13</td>
</tr>
<tr>
<td>Gastric carcinoma</td>
<td>3-11</td>
</tr>
<tr>
<td>Esophageal carcinoma</td>
<td>5-7</td>
</tr>
</tbody>
</table>
**Table 2.** Distribution of skeletal metastases. Reproduced with permission from Galasko, 1981(15). Copyright Wolters Kluwer journals.

<table>
<thead>
<tr>
<th>Type of Carcinoma</th>
<th>Mammary</th>
<th>Prostatic</th>
<th>Thyroid</th>
<th>Misc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>Dorsal vertebrae</td>
<td>Lumbar vertebrae</td>
<td>Skull</td>
<td>Vertebrae</td>
</tr>
<tr>
<td></td>
<td>Lumbar vertebrae</td>
<td>Femur</td>
<td>Vertebrae</td>
<td>Ribs</td>
</tr>
<tr>
<td></td>
<td>Ribs</td>
<td>Pelvis</td>
<td>Pelvis</td>
<td>Skull</td>
</tr>
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<td></td>
<td>Pelvis</td>
<td>Dorsal vertebrae</td>
<td>Ribs</td>
<td>Vertebrae</td>
</tr>
<tr>
<td></td>
<td>Proximal femur</td>
<td>Skull</td>
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<td>Pelvis</td>
<td></td>
<td>Sternum</td>
</tr>
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</table>

**Figure 1.** Osteoclasts and osteoblasts in normal bone and bone Metastasis. (a) Osteoclasts and osteoblasts in normal bone (toluidine blue, x100). The large osteoclast is actively resorbing bone. Osteoblasts are small, cuboid cells that actively lay down bone matrix. (b) Osteolytic bone metastasis (hematoxylin and eosin, x200). Renal carcinoma cells are invading the bone marrow, and osteoclasts (arrows) are actively resorbing bone adjacent to the tumour cells. (c) Osteoblastic metastasis (hematoxylin and eosin, x200). Thickened trabeculae and large numbers of osteoblasts are present next to the bone surface. Tumour cells from adenocarcinoma of the lung are seen between the two large trabeculae. Reproduced with permission from Roodman, 2004 (16), Copyright Massachusetts Medical Society.

In both breast and prostate carcinoma, a valve-less venous system, Batson’s plexus, connects the deep spine and pelvis to the breast and prostate. The slow flow through this venous network can allow retrograde flow, facilitating migration of the malignant cells to the bone (6). In the axial skeleton, the most common site of bone metastases, red marrow has been identified to encourage tumourigenesis. Since red marrow possesses sinusoids, or small blood vessels with discontinuous endothelium, it allows unrestricted flow of molecules across its thin walls, facilitating dissemination of the cancer cells and ultimately metastases (5, 6).
From a molecular perspective, a cascade of interactions of the bone’s constituents contributes towards the development of metastatic disease. Tumour cells produce adhesive molecules that bind them to marrow stromal cells and bone matrix. The resulting interactions give rise to increased production of angiogenic factors and bone resorbing factors that further induce tumour growth in bone (16).

1.1.1 Bone Tissue, Remodeling and Metastases

Bone is a highly specialized, active and dynamic organ composed of three main cell types: osteoblasts, osteoclasts and osteocytes. Other components of bone include mineralized and unmineralized connective tissue matrix, bone marrow cavity, vascular canals, canaliculi, and lacunae (17). Osteoblasts, the small mononuclear cuboid cells that originate from the multipotent mesenchymal stem cells of the marrow, are responsible for producing osteoid and laying down bone matrix (18). Osteoclasts, the larger multinucleated cells, originate from hematopoietic progenitors in the bone marrow (similar to monocytes and macrophages) and actively resorb bone (19). Osteocytes, are the stellate shaped mature osteoblasts buried within lacunae of mineralized matrix that are considered the mechanosensory cells of bone tissue. Osteocytes are the most prevalent cell type which communicate with each other and the bone surface cells via their extensions along the canaliculi and control the bone remodeling process (20).

Bone remodeling, inclusive of bone resorption and formation, is composed of basic multicellular unit (BMU) which in the healthy adult is approximately 1-2 mm long and 0.2-0.4 mm wide (17). BMU consists of a team of osteoclasts in front and a team of osteoblasts in the back, as well as a central vascular capillary, a nerve supply, and associated connective tissue. The lifespan of the BMU is 6-9 months, though its executive cells will only have a ~2 weeks (osteoclasts) or ~3 months (osteoblasts) lifespan. In the cortical bone (the dense structure of the outer shell of most bones) BMU travels through the bone and creates and replaces a tunnel (harversian system). In trabecular bone (found at the end of long bones or inside the vertebral body) the BMU moves across the trabecular surface creating and replacing a pit (17). Bone remodeling is both a local and systemic phenomenon and is highly important in adjusting bone architecture in response to mechanical needs, microdamages, prevention of accumulating old bone, and maintaining plasma calcium homeostasis. A number of systemic regulators involved in the remodeling process
comprise parathyroid hormone (PTH), calcitriol, growth hormone, glucocorticoids, thyroid hormones, sex hormones, insulin-like growth factors (IGFs), prostaglandins, transforming growth factor-beta (TGF-β), bone morphogenic proteins (BMP), and cytokines. Additionally, the receptor activator of nuclear factor-kappa B (RANK)/ligand (RANKL)/osteoprotegerin (OPG) systems locally control the bone resorption/formation processes (21).

In a metastatic situation, tumour-produced factors will stimulate either osteoclasts or osteoblast activity and disrupt normal bone remodeling. The tenacity with which tumour grows in bone is dependent upon the tumour/bone cell interactions as well as the fertile soil microenvironment of the bone which will also dictate the osteolytic or osteoblastic phenotypes. In both phenotypes, the tumour/bone cell interactions constitute a vicious cycle where tumour cells stimulate the respective bone cells to resorb or form new bone. This results in the bone microenvironment producing more growth factors which will in turn further fuel tumour growth in bone. Active protagonists in the osteolytic metastasis include tumour-produced PTH-related protein (PTHrP) and bone-derived TGF-β. In osteoblastic metastasis a large concentration of immobilized growth factors have been suggested to stimulate disorganized new bone formation. These include: TGF-β, insulin-like growth factors (IGF) I and II, fibroblast growth factors (FGF) I and II, platelet derived growth factors (PDGF), bone morphogenetic proteins (BMPs), endothelin (ET) I and calcium (16, 22).

1.1.2 Biomechanics of Metastatic Vertebrae

The development of pathological fracture in metastatically involved vertebrae can occur due to traumatic loading or under normal physiological stress (13, 23). Osteolytic metastases in particular, weaken the vertebral structure and cause internal pressurization under loading. Increased surface tensile hoop strains, and bulging and spinal canal narrowing can occur even under low loading levels, leading to an elevated risk of pathologic burst fracture (failure of the posterior wall of the vertebral body) (24). Factors such as the size and location of the tumour, the extent of tumour destruction, increased load, pedicle involvement, and the patient’s bone mineral attenuation and bone mineral density, can determine how and when pathologic fracture (including pathologic burst fracture) occurs (24, 25). Biomechanically, destruction of the middle third of the vertebral body in the axial plane due to tumour will result in gross instability, whereas destruction of the middle third in the sagittal plane will not be associated with
significant destabilization (26). In the presence of intact posterior elements, ventrally-situated tumours will have a greater potential for destabilization (26).

The ability of the spinal column to maintain its pattern of displacement under physiological loading is the accepted definition of spinal stability from a mechanical perspective (27). In this context, vertebral mechanical strength and integrity play a role in maintaining overall spinal stability. The definition of spinal stability from a clinical perspective, however, includes mechanical stability as well as absence of any neurologic damage, deformity or pain (27). The mechanical stability of the vertebral body incorporates both its resistance to deformation in both the axial and radial directions, which includes movement of the posterior vertebral body wall and ultimate strength (see also Chapter 5).

**Figure 2.** Lumbar vertebral anatomy. Adapted from "Spine Centre"(28)

### 1.2 Imaging for Bone Metastases

The most common imaging modalities for bone metastases are bone scans ($^{99m}$Tc bone scintigraphs), x-ray radiography, Computer Tomography (CT), and Magnetic Resonance Imaging (MRI). Imaging is used to detect bone metastases as early as possible, identify the extent of the disease, identify complications (i.e. SREs), monitor response to therapy, and
sometimes guide local therapies or biopsy needles in the tissue (29). Bone scans are cost effective and have shown adequate diagnostic accuracy comparable to MRI (30). Based on the nature of the disease, the radiographic evidence of the tissue may appear lytic, sclerotic (blastic), or mixed. Lytic destruction of bone or significant sclerosis in radiologic imaging have been quoted as obvious signs of metastases (31). Conventional x-ray radiography is appropriate for imaging such abnormalities but provides minimal data on the integrity of the marrow. Additionally, x-ray imaging may only detect metastases at relatively late, more evolved stages (i.e. 30-70% bone density loss). As such, occult metastases may be present without x-ray radiographic evidence. Similarly, false positive or imposter lesions may be detected where abnormalities are seen on the x-ray images of patients with known primary tumours. CT is advantageous due to its higher resolution, soft-tissue contrast and detailed morphology. CT scans have been reported to be sensitive in detecting bone metastases in 70%-100% of cases. The drawback for CT imaging is its low sensitivity for early detection of malignant bone involvement, as it requires substantial changes in the bone for visualization. Also, if the bone is already degenerative or osteoporotic, or if the marrow is infiltrated, CT imaging may be inadequate for diagnosis. MRI allows visualization of bone marrow and metastatic disease at an early stage with relatively good spatial and contrast resolution. The limitation of MRI is its lower sensitivity for detection of bone destruction and differentiation between active disease, scar, necrosis or fracture when monitoring the tissue post treatment (29, 31). For instance, in MR imaging of osteoid osteomas, the signal in the nidus is often similar to muscle on T1-weighted images, but variable on T2-weighted images. Hyperintensive signal is seen in the lesion's reactive zone on T2-weighted or short inversion time inversion-recovery images. However, imaging features may be similar to those of a stress fracture or osteomyelitis (particularly in presence of surrounding edema) and nonspecific (32). Similarly, fat-suppressed inversion recovery sequences have been indicated to be useful in detection of bone metastases but their role in evaluation of therapeutic response has been controversial (33, 34). Dynamic imaging with use of gadolinium-based contrast enhancement material may provide increased clarity and more accurate diagnosis (35)(36).

Functional imaging, including Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) are more recent advances in imaging technology with high specificity. $[^{18}F]$ fluoride PET and FDG PET are particularly useful in distinguishing
lytic bony abnormalities and osteoblastic activities respectively (29). SPECT imaging has been shown to be more sensitive in detecting spinal malignancies than planar bone scans (37). Integrated techniques such as PET/CT and SPECT/CT combine morphologic and functional studies and are becoming more widely available in routine practice (29).

1.3 Histology for Bone

Histology is a common and reliable method for examining bone pathologies and confirming diagnoses. Clinically, a biopsy sample is usually acquired and processed for microscopic evaluation, while experimentally, an entire bone organ, such as a vertebral level or an appendicular fragment, can be processed for analysis. Processing of osseous tissue for histological preparation is generally more complicated than soft tissue based on the inherent properties of bone and its mineralization. Specimens are first fixed, most commonly in 10% neutral buffered formalin, which permits use of a large number of stains. Samples may be prepared for sectioning, fresh or fresh-frozen and then ground in order to maintain the bone tissue ‘as-is’, leaving fewer options for staining. They can also be decalcified using a number of various solutions (such as hydrochloric acid, formic acid or ethylene diamine tetraacetic acid (EDTA)) and then embedded in paraffin or resin (38). Resin embedding is generally more time-consuming and a gentler technique. Sectioning of the specimens, which follows embedding, may influence the usefulness of the samples. In general, thin sections are necessary for analysis of cellular detail and thick sections for lower-magnification histomorphometric studies. Several staining methods are available for bone analysis. Hematoxylin & eosin (H&E) staining is the most basic and common technique, consistent with soft tissue, and can be used for both decalcified and calcified specimens. Goldner’s trichrome and von Kossa stains can identify osteoid from mineralized bone matrix in calcified bone samples. Tetracycline labeling, administered during an experiment and before animal sacrifice, is used to monitor bone formation and rate of remodeling in calcified bone samples. Alkaline phosphatase, an ectoenzyme present in the osteoblast and in matrix vesicle membranes, and tartrate-resistant acid phosphatise (TRAP), a lysosomal enzyme whose localization provides a sensitive method of osteoclast identification, are two phosphohydrolases used for osteoblast and osteoclast identification respectively. Immunohistochemical staining can identify biochemicals such as glycoproteins, laminin, tenasin and fibronectin in resin-embedded specimens. A challenge in
bone histology is that multiple parameters of interest often cannot be obtained with one staining procedure or localization method (39).

1.4 Current Therapies

There are multiple approaches in the treatment of bone metastatic disease, including systemic and local therapies. With improvements in cancer therapies, patients are living longer even with established bone metastasis, increasing the need for treatment (5, 40). The treatment regimen for spinal metastasis is generally palliative with a therapeutic goal to improve patient’s quality of life by alleviating pain, structurally stabilizing the spine and preventing neurological injury or, when necessary, improving lost neurological function. The exact mechanism of pain from osseous metastases is not fully understood; however, theories include increased pressure on and stretching of the periosteum and microfractures, as well as release of bradykinin, prostaglandins, substance P, and histamine (41). A brief review of the common available treatment strategies follows.

1.4.1 Radiation Therapy

Radiation therapy is commonly used in the treatment of metastases and aims to palliate pain and prevent tumour recurrence / growth (8). With conventional external beam radiation therapy (XRT), approximately 70% of patients experience pain relief between 2-3 days and up to four weeks post treatment (42). However, radiation therapy may also create complications, due to damage of neighbouring soft tissues (42). Pain treated by radiation therapy returns in 54% of cases on average in less than four months (43). Radiation therapy is also limited by tissue tolerance and toxicity levels. Therefore, radiation of a previously irradiated metastatic site may not be possible (2). In general, some tumours are very sensitive to radiation therapy (i.e. lymphoma, myeloma, neuroblastoma, Ewing’s sarcoma and seminomatous germ cell tumours). Others are intermediately radiosensitive (i.e. most solid tumours including breast cancer, prostate cancer, and lung cancer). Alternatively, tumours may be radio-resistant (i.e. melanoma, osteosarcomas, thyroid, colon and renal cell carcinomas) (8). A newer radiation intervention, Stereotactic Body Radiation Therapy (SBRT), can accurately deliver a large ablative dose to a more targeted area of bone. There is much enthusiasm associated with this new method as it may be a viable treatment strategy for radio-resistant tumour cells (44). SBRT has been applied to
spinal metastases, but it has been noted that a high incidence of fracture occurs in individuals treated with this approach (45, 46). Examples of XRT dosing for symptomatic vertebral lesions involve 8 Gy in 1 fraction (low), 20 Gy in 5 fractions, to 30 Gy in 10 fractions (42). In contrast, SBRT may apply 24 Gy in 1 to 2 fractions to 30-40 Gy in 5 fractions (47). No regimen has been shown to be significantly more effective than the other in XRT and SBRT (42, 47).

Radiation therapy is limited in that pain relief may be delayed up to two weeks post-procedure in some patients and this treatment does not address existing skeletal instability. Radiation therapy is ineffective in preventing impending vertebral body collapse (which up to 50% of patients receiving the treatment experience). As such, it has been suggested that radiation therapy may prove most effective as an adjuvant post stabilization procedure (13, 43). A clinical example motivating a multi-modality approach in therapy is highlighted by Patchell et. al.’s 2005 randomized control trial which demonstrated that surgery in combination with radiation therapy versus radiation therapy alone was more effective in maintaining ambulatory capacity in individuals presenting with metastatic epidural spinal cord compression (48).

1.4.2 Surgical Approaches

Metastatic bone tumours in the vertebrae are painful and debilitating and often require challenging and extensive surgeries that many clinicians and patients are reluctant to perform (13). Open procedures range from wide en-bloc total resection for carefully selected patients with oligo-metastatic tumours to intra-lesional debulking procedures that decompress neural elements in patients that are symptomatic. Other goals of surgery also include reduction of spinal deformity and improved stabilization. Surgery may involve resection of individual vertebral bodies, discs, anterior and posterior longitudinal ligaments, and the dura in order to provide adequate tumour margins (wide en-bloc excision). Occasionally neural, vascular and muscular structures may also need to be sacrificed. Depending on the location of the tumour, surgery may only be marginal or intra-lesional at best. As such, the decision to undertake surgery may be outweighed by the associated risks (49). Achieving sufficiently wide margins without contamination is also a challenge in surgical resection; therefore, surgery may be combined with a secondary modality, such as radiation therapy with a goal to maximize reduction of local tumour cell viability. In this however, the negative impact of radiation therapy on wound healing is an important consideration. Less invasive methods, such as ‘tubular’ surgical systems (i.e. low
profile radiolucent retractors inserted through tissue sparing surgical approaches to access the surgical area of interest) may also reduce the morbidity associated with open surgery in carefully selected patients.

1.4.3 Percutaneous Image-guided Vertebral Body Augmentation

Minimally invasive surgical approaches for augmentation of the vertebrae (vertebroplasty or balloon kyphoplasty) are becoming increasingly common in the treatment of metastatic spinal disease. The idea of pain reduction using bone cement augmentation was first introduced by Harrington in 1981. His method was essentially a “fusion” type of procedure where, after removing portions of the tumour, bone cement was inserted around and into the tumour-affected vertebral body to stabilize the spine (50). However, the first report of vertebroplasty was published by Galibert et al. in 1987, where patients suffering from painful vertebral hemangiomas showed improved results (51).

Vertebroplasty involves the injection of liquid polymethylmethacrylate (PMMA, bone cement) through a bone trochar needle inserted percutaneously into a targeted vertebra. Solidification of the PMMA occurs through an exothermic reaction (52, 53). Balloon kyphoplasty involves inserting an angiocatheter-like device through the bone trochar that is inflated to create a void which is then filled with cement. In theory, inflation of the balloon may help improve sagittal plane spinal alignment (54).

Percutaneous vertebral augmentation of metastatic spines can be technically challenging since many patients have epidural extension of the tumour with possible disruption of the posterior cortical border. Cement extravasation is the most common complication of vertebral augmentation. Although, approximately 90% of the patients receiving vertebroplasty show improved pain scores, cement extravasation occurs in ~41% of treated vertebrae (55). Complications resulting from bone cement extravasation occur more frequently in treatment of metastatic patients (≤ 10%) than in those with osteoporosis (1-2%) or spinal angiomas (2-3%) (56). When cement extravasation occurs in the intradiscal space, it has been found that adjacent level fracture is more likely to ensue (57). The mechanism of pain relief for cement augmentation is claimed to be associated with stabilization of microfractures, destruction of pain receptors and stabilization of the motion segments (8). There has been controversy more recently
on the role of vertebral cement augmentation for vertebral fractures (i.e. those relating to osteoporosis/osteopenia/vertebral insufficiency fractures) given results from recent randomized control trials (58). The use of these approaches in skeletal metastases, however, remains widely accepted, as fractures in such cases tend not to heal when compared to fractures due to osteoporosis/osteopenia (59). A recent randomized trial (Berenson et al., 2011) demonstrated that painful vertebral compression fractures in patients with cancer could be improved with balloon kyphoplasty when compared to non-surgical management (59).

In some cases minimally invasive procedures may also be used concurrently with an open procedure to reduce invasiveness. The combination of posterolateral spinal decompression with cementoplasty (Kyphoplasty™ or vertebroplasty) can be performed through smaller surgical approaches when compared to conventional spinal surgery (8).

1.4.4 Bisphosphonates and Other New Drug Therapies

Bisphosphonate therapy is a current standard of care for osteolysis-related pathologies from any solid tumour or multiple myeloma, particularly if there is a significant risk for morbidity. There are a number of bisphosphonates clinically available or in the trial phase, each of which targets a specific mechanism in the bone (60). Bisphosphonates impact the function of osteoclasts by inhibiting their recruitment, diminishing their life span, and inhibiting their activity at the bone surface (61, 62).

Structurally, bisphosphonates are similar to endogenous proton pump inhibitors (PPi) and have a P-C-P backbone and two covalently bonded side chains, R₁ and R₂. The P-C-P backbone and R₁ side chain express a strong binding affinity to the hydroxyapatite found in the bone, which lead to accumulation of bisphosphonates in the regions of increased bone activity. The R₂ side chain establishes potency of the particular bisphosphonate to inhibit osteoclast activity (60). A number of bisphosphonate therapeutic agents are currently available, some of which include: Clodronate (which lacks a nitrogen functional group in its R₂ chemical structure), Pamidronate, Ibadronate, Risendronate, Zoledronic Acid (FPP inhibitor) and Minodronate (all of which are nitrogen-containing bisphosphonates) (60).

Bisphosphonates are potent antiresorptives that, in addition to inhibiting the osteoclast-mediated bone resorption, reduce the local growth factors and slow down tumour cell growth. They also
induce tumour cell apoptosis and inhibit tumour cell adhesion, invasion, and angiogenic activity. However, although bisphosphonates increase the volume of bone, in many cases they do not completely prevent or remedy bone metastases (14). It has also been suggested that bisphosphonates may play an unclear role in some primary malignancies (2). Another concern is that by inhibiting bone turnover, bisphosphonates therapy can lead to damage accumulation within the bone. Side effects of bisphosphonates include renal complications, atypical femoral stress fractures, and osteonecrosis of the jaw (61).

Other drug therapies are also available. Denosumab (a RANK-RANKL inhibitor) is a human monoclonal antibody that binds and neutralizes RANKL, a key mediator in the metastatic bone destruction cycle and, in combination with Zoledronic Acid, has been prescribed to manage osteoblastic prostate metastases (63). Odanacatib (Cathepsin K inhibitor) suppresses bone resorption by inhibiting the osteoclastic cysteine protease, cathepsin K. Atrasentan (Endothelin receptor antagonist) binds to and inhibits its receptor ET-A and has been shown to block osteoblastic metastases. Dasatinib and Sarcatinib (Src kinase inhibitors) suppress the key resorptive osteoclastic Src molecule and reduce lytic metastases (7).

1.4.5 Chemotherapy

Chemotherapy is not widely used in the treatment of symptomatic spinal metastases; however, it may be administered combined with radiotherapy and/or surgery or as an adjuvant treatment post radiotherapy or surgery. Indications for chemotherapy are limited. It has been shown to be effective only in chemosensitive tumours of the spine, such as lymphoma, some pediatric tumours (i.e. neuroblastoma) and seminoma (64). Hoshi et al. reported a case of aggressive bone metastasis as a result of sarcomatoid renal cell carcinoma that was effectively treated with chemotherapy (65). Evaluation of other types of bone metastases such as breast cancer bone metastases treated with chemotherapy has only shown partial improvement in 67% of patients (66).

1.4.6 Thermal Therapies

Thermal ablation therapies for bone metastases have a collective long history. In general, ablation refers to the destruction of a tissue by means of local application of chemical agents (i.e. ethanol, acetic acid), or local deposition of some form of energy (radiofrequency, laser,
microwave, ultrasound or cryoablation)(41). All thermal ablation modalities share a focal and targeted therapeutic style and function by heating or cooling the region of interest with the objective of producing cell necrosis.

The concept of using heat as a treatment modality is not new. Hippocrates has been quoted with the statement that “an illness not cured by heat is incurable”(67). As early as the 1800s, physicians were using heat to treat cancer (68). It is therefore not surprising that many of the thermal therapies are based on tissue heating. In all the available heating procedures, the goal is to raise the tissue temperature to between 60-100 °C and produce coagulative necrosis, while minimizing charring, boiling or iatrogenic injury (69).

a) Microwave Ablation

Microwave ablation is a thermal modality that operates with 14.5 G antennae, sending electromagnetic waves between 900-2450 MHz. Heating of the target tissue is based upon agitation of water molecules that induce cellular death via coagulative necrosis. Specifically, the electrical charges on the water molecules flip back and forth at a rate of 2-5 billion times per second depending on the microwave frequency range. Theoretically, microwave ablation should be uniform and able to target larger lesions, as it is not dependent on the tissue impedance. With microwaves it may be possible to use multiple applicators at once or to heat cystic masses and tumours near blood vessels or without charring. The literature suggests that there is currently only one microwave ablation system (Evident TM Microwave ablation system/Covidien Ltd) commercially available. Other systems in the literature have been referred to as experimental apparatuses made in house. Lack of widespread availability of microwave ablation systems renders clinical employment of this technique for bone metastasis infrequent and the related literature scarce (2, 70-72).

b) Laser Ablation

Laser ablation works by sending infrared light energy to the tumour through thin optical fibers of 400-600 μm. The laser energy heats the tissue and creates coagulative necrosis. Two types of neodymium yttrium aluminum garnet (NdYAG λ 1064nm) and solid state lasers (λ 805 nm) have been successfully used in tumour ablation. Fiber arrangements may change based on the treatment area and may result in a spherical or elliptical zone of treatment. Laser ablation has
been mainly used in treatment of osteoid osteoma and is capable of ablating smaller regions. There is very limited experience with laser ablation in treatment of bone metastases (73). An advantage of laser ablation is the compatibility of the device with MRI that facilitates localizing the target (2, 69, 74). The limitation of this technique is small regions of effect and long treatment durations (i.e. 60-90 min). Additionally low levels of pain reduction have been clinically reported (75).

c) **Focused Ultrasound Ablation**

Focused Ultrasound (FUS) ablation has been available since the 1940s but recently there is much renewed interest in FUS due to advancement in technology. FUS is a non-invasive thermal therapy that uses extracorporeal-focused ultrasound energies of 0.8-3.2 MHz and focal peak intensities of 5000-20000 W/cm² to penetrate bone, heat and destroy tumour lesions without intrusion of applicators. The mechanism of function in FUS is based on bone’s acoustic absorptive properties and the heating can work to destroy periosteal innervation, palliate pain and ablate tumour tissue. FUS can be operated under MRI guidance and MRI-based thermometry determines the dose of energy deposition. Also, since this is a trackless method, there is no associated risk of tumour seeding. The main disadvantage of FUS may be the limited amount of necrosis that can be achieved per unit of time, which can lead to a long procedural time in each session (2, 69).

A more recent anti-tumour application of FUS concerns its use in combination with microbubbles which are traditionally used as contrast agent in ultrasound imaging. These ultrasound activated micron sized, gas bubbles, typically surrounded by a biocompatible protein or lipid layer, have been shown to have arteriogenesis, angiogenesis and neovascularization properties in mouse skeletal muscle (76, 77). As well, microbubbles have been exploited to mechanically damage the tumour vasculature endothelial layer using low dose non-invasive ultrasound radiation which can be utilized to enhance effects of radiation therapy by lowering the required dose for treatment (78, 79).

d) **Cryoablation**

Cryoablation relies on freezing and thawing of the tissue to generate necrosis. Historically nitrogen gas was used to cool and kill tumour cells, but recently Argon, delivered through a
partially-insulated probe, is used in these procedures. The gas undergoes a rapid (Joule-Thompson) cooling, and can lower the local temperature to -100 °C in a few seconds. Ice can penetrate bone and therefore achieve a good ablative coverage of osteoblastic metastases. Following the rapid freezing cycle, Helium gas is delivered to the site to warm the tissue and to remove the probe. Cryoablation probes and the ice-ball tip are easily visible under MRI or US imaging and the margin of ablation is relatively obvious. The probes may be as small as <2.5 mm in diameter and can come in different sizes and shapes. Cryoablation generally does not induce immediate or post-operative pain in the patients (2, 69, 73, 74). One drawback of cryoablation is the sudden release of tumour cellular contents as a result of thawing the frozen tissue, which can lead to a potentially serious condition referred to as cryoshock (80).

e) Radiofrequency Ablation

Radiofrequency Ablation (RFA) is the most widely adopted thermal ablative method. It will be discussed in detail in the following sections. RFA is most effective in thermally ablating small soft tissue lesions away from large vascularisation that may act as a heat sink. RFA is technically simple to use, with several commercially available devices to choose from. It is particularly useful when surgical resection is technically unfavourable. It has been clinically used in various tissues and shown to significantly reduce pain within one week of treatment (2). The main shortcoming of RFA is its inability to comprehensively necrotize large tumours. As well, due to potential electrical interference, its use in patients with pacemakers has been cautioned against (81, 82).

1.5 Radiofrequency Ablation

1.5.1 Overview

Radiofrequency (RF) refers to the electromagnetic (EM) spectrum covering the frequencies from 3 Hz to 300 GHz. In medicine, applied RF waves cause thermal ablation of a defined volume of tissue (83). RFA has historically had medical applications in pain management and cardiology. The first functional commercially available RF generators were built in the 1950s and used continuous wave RF in the 1-MHz range (84). In general, analogous apparatus, with varying electrode specificities, have been used to ablate nerve endings of problematic structures such as vertebral levels (for management of back pain) or facial nerves to relieve jaw pain. Similarly, in
cardiology, RFA has been used to treat conditions such as atrio-ventricular septal defects (85, 86). In oncology, use of RF energy began over 50 years ago with the treatment of small cerebral tumours (Aranow, 1960) and has since become a common and multi-organ therapeutic option. Depending on the tissue, the goal of RFA is to ablate a margin of 0.5-1.0 cm of normal tissue, consistent with a surgical margin. This would equate to ablation of approximately 3-4 cm for a 2 cm tumour. RFA is most widely used in hepatic tumour treatments and soft tumour tissues. More recently bone tumour treatment has also become a medical application of RFA. However, up to one third of patients undergoing RFA for a tumour treatment have been quoted to experience post-RFA syndrome, symptoms of which may include, a low-grade fever, malaise, myalgia, delayed pain, nausea and vomiting. The symptoms are considered self-limiting and expected to resolve within 10 days post-procedure (87).

RFA requires active electrode(s), a ground electrode, and a radiofrequency generator. The needle-like electrode(s) or probe is thin, usually 21-14 gauge, allowing a stereotactic technique. It is a metal shaft that is insulated throughout, leaving an exposed conductive tip, which comes in contact with the tissue. A RF generator supplies the alternating current (AC) through the electrode and controls the ablation based on a feedback system dependent on tissue impedance, tissue temperature, or power output control. The generator is connected both to the electrode and the grounding or dispersing pad. The grounding pad is often a large-area electrode that is not intended to induce tissue heating and may be positioned on large muscle groups such as the patient’s thigh (Figure 3). RF needle placement is generally achieved using US, CT, Fluoroscopy or MR imaging guidance (84, 88).
Figure 3. The RF ablation “circuit”. The electrode acts as the cathode, and the pad as the anode. The patient is actually part of the circuit, and tissue conductivity is important in achieving adequate ablation zone.

1.5.2 Mechanism of Function of RFA

a) Physics

At the frequency range of medium waves (300-3000 KHz), RF energy does not stimulate or interfere with neuromuscular reaction and electrolysis and is sufficient to confine energy transmission to a relatively controllable tissue mass without excessive radiation (89).

The basic RFA circuit, shown in Figure 3, has four main components connected in series: (1) the RF generator which is the source of RF voltage across its output terminals, (2) the electrodes which when connected to the generator allow flow of the current to the target tissue, (3) the patient’s body which becomes an active element of the electric circuit and facilitates the passage of the electrons, and (4) the ground or dispersive electrode which closes off the circuit, generally, without inducing tissue heating around itself. With all the components in place, and the generator turned on, the target tissue induces an impedance, and a high flux is created around the small cross-sectional area of the electrode tip (also known as the active tip), and dispersed by the grounding pad or electrode. This mechanism ensures that the tissue damage (or ablation) is confined to the active electrode tip (83, 84).

In the tissue, the dipole molecules (mainly H₂O) immediately next to the active tip of the electrode align in the direction of the current and are forced to vibrate as rapidly as the alternating current is applied. Farther molecules are then set into motion by adjacent vibrating
molecules. The frictional energy, created as a result of these vibrations, leads to a rise in temperature, which is responsible for the coagulative necrosis of the targeted tissue. As such, the electrode itself is not hot or the source of heating, rather it functions to generate an EM field that sets an ionic agitation in the target tissue that leads to heating (83). Therefore, thermal and electrical conductivity of the tissue is imperative to successful ablation. If, for instance, the tissue becomes desiccated or charred as a result of the applied current, it can no longer serve to vibrate (and thus heat) the neighbouring molecules and an ineffective procedure may ensue (83). For this reason, one criterion in design of RFA devices is a slow methodical temperature rise, which will improve the outcomes of the procedure. In cases of rapid temperature rise, or temperatures greater than 105 °C, boiling, vaporization and carbonization may occur, resulting in lowered energy transmission and small or incomplete ablation zones.

From a conceptual framework, Goldberg et al. described the following relationship regarding development of a thermal lesion in the target tissue via RFA (88):

Eq. 1  \[ \text{Induced coagulation necrosis} = (\text{energy deposited} \times \text{local tissue interactions}) - \text{heat loss} \]

Liu et al., later used the formal Bioheat equation (Eq. 2) to show the potential importance of power, thermal conductivity, and perfusion on ablation outcomes.

Eq. 2  \[ \rho c_t \frac{\partial T(r,t)}{\partial t} = \nabla \cdot (k_t \nabla T) - \rho_b c_b \rho m \rho_t (T - T_b) + Q_p(r,t) + Q_m(r,t) \]

where,

\( \rho \) = density of tissue, blood (kg/m\(^3\)),

\( c \) = specific heat of tissue, blood (Joules/kg\(^\circ\)C),

\( k \) = thermal conductivity,

\( m \) = perfusion (blood flow rate/unit mass tissue) (kg/m\(^3\) sec),

\( Q_p \) = power absorbed/unit volume tissue,

\( Q_m \) = metabolic heating/unit volume of tissue,
In addition, the electrostatic equation (Eq. 3) shows the importance of current density and electrical conductivity of the tissue on RF-induced tissue heating (90).

Eq. 3 \[ Q_p = j^2/\sigma \]

where,

\[ J = \text{current density}, \]
\[ \sigma = \text{electrical conductivity}, \]

Computer modeling findings generated using these equations confirmed that the electrical conductivity of the tumour and surrounding tissue, thermal conductivity of the tissue, the tissue perfusion, and radiofrequency generator output are important parameters that should be accounted for in device design (90).

\textit{b) Biology}

As previously described, the frictional heating resulting from the vibration of the ionic constituents of the target tissue will increase the local temperature and result in coagulative necrosis. It has been stated that temperatures above 42 °C will induce cell death in some tissues. Cells (both cancerous and healthy) in the range of 41-47 °C begin to show signs of apoptosis (68). Others have noted that in the temperature range of 42-45 °C, cells become more susceptible to damage by other agents such as chemotherapy and radiation therapy, but do not undergo cell death even at prolonged heating at these temperatures (91). The plasma membrane potential has been shown to be affected by heat beyond 43 °C upon prolonged exposures (92). Nevertheless, there is a collective agreement that when temperatures above 60 °C are present, the cell begins to undergo frank necrosis rather than apoptosis (9, 87). Cell necrosis, whether from heat, strong acid or base, is the result of protein denaturation, which follows “melting” of the lipid bilayer and the cells falling apart. The instantaneous protein coagulation resulting from such high temperatures irreversibly damages key cytosolic and mitochondrial enzymes, as well as nucleic acid-histone protein complexes (91). In contrast, apoptosis requires protein creation mechanisms to be intact. Several types of pharmacologic or biologic agents (i.e. “death-inducing signaling complexes”) activate Caspase-8 and cause apoptotic cell death in an organized order. Injurious
stimuli, such as heat or cold, can induce pro-apoptotic proteins to induce Caspase-9. Caspase-8 or -9 expressions initiate a cascade effect that eventually leads to cell death (68). Beyond 100 °C, the intra and extracellular water in the tissue boils and vaporizes and leads to carbonization of the surrounding tissue. In RFA context, this also serves as an electrical insulator which prevents further heat deposition and ablation (93).

A typical radiofrequency ablated region, may be composed of several zones undergoing frank necrosis, moderate necrosis, apoptosis and moderate apoptosis, based on distance from the electrode. Additionally, the Heat-Shock proteins (HSPs) within the cells, may render some cells viable after low intensity, repeated, heat treatment (68).

1.5.3 Devices

A large number of RF devices are currently available in the market (Table 3, Figure 4). Almost all medical generators today use a frequency range of 450-600 KHz for ablation. Probe design, circuitry, and feedback mechanism are the main differences that distinguish these devices. The devices may be monopolar or bipolar (depending on the grounding electrode design), conventional, internally cooled or perfusion (to allow release of saline or water), single needle or multi-tined (modification of active electrode tip design) or a combination of the above. A brief review of the various available devices will be presented below.

a) Monopolar Probes

In monopolar RFA of a tissue, an electrical circuit is created where the electrode acts as the cathode, and a dispersing or grounding pad (which is usually placed on the patient’s thigh), acts as the anode and closes off the circuit. Most initial designs of RFA probes used in neurological denervation are monopolar.
### Table 3. Summary of some of the commonly cited RF probes in the literature

<table>
<thead>
<tr>
<th>Probe Type</th>
<th>Commercial Name</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monopolar</strong></td>
<td>Titanium RF electrode</td>
<td>Radionics, Burlington, MA, USA</td>
</tr>
<tr>
<td></td>
<td>Arthrex AR-9603-60 ablation probe</td>
<td>Bovie Medical Corporation St. Petersburg, Florida, USA</td>
</tr>
<tr>
<td></td>
<td>TCM 101 Conventional monopolar needle electrode</td>
<td>Stryker Leibinger, Freiburg, Germany</td>
</tr>
<tr>
<td><strong>Bipolar</strong></td>
<td>Arthrocare System 2000</td>
<td>Arthrocare Corp, Sunnyvale, CA, USA</td>
</tr>
<tr>
<td><strong>Internally Cooled</strong></td>
<td>Cool-Tip electrode</td>
<td>Covidien, Boulder, Colorado</td>
</tr>
<tr>
<td></td>
<td>Cluster electrode</td>
<td>Radionics, Burlington, MA, USA</td>
</tr>
<tr>
<td><strong>Perfusion</strong></td>
<td>XLI-Enhanced and Talon electrodes</td>
<td>AngioDynamics, Queensbury, New York</td>
</tr>
<tr>
<td><strong>Multi-tined</strong></td>
<td>LeVeen electrode</td>
<td>Boston Scientific, Nantucket, Massachusetts</td>
</tr>
<tr>
<td></td>
<td>RITA Starburst XL</td>
<td>RITA Medical Systems, Inc., Mountain View, CA, USA</td>
</tr>
</tbody>
</table>

**Figure 4.** Examples of various RFA electrodes

**b) Bipolar Probes**

Traditionally, in bipolar RFA two electrode applicators are placed in proximity to achieve contiguous coagulation between the two electrodes. The active electrode supplies the RF energy
while the dispersive electrode serves as the return path (94). Less current is required in a bipolar setting compared to a monopolar probe to achieve the same effect simply because the current passes through a much smaller volume of tissue. Bipolar RFA produces oval-shaped lesions (as compared to spherical lesion of the monopolar designs (95)) and has generally been shown to produce larger lesions compared with monopolar ablation using a single-needle electrode. Limited coagulation between the bipolar electrodes has also been demonstrated and is known to be a function of the distance between the electrodes (94, 96).

c) Internally Cooled Probes

Internally cooled electrodes, (i.e. Cool-Tip electrode (Covidien, Boulder, Colorado) and Cluster electrode (Radionics, Burlington, MA, USA)), use an interior lumen, filled with a circulating liquid, to remove the heat from the tip of the electrode and enhance the size of the ablation zone, while minimizing charring and impedance in the tissue. Internal cooling of RFA electrodes is considered a technological advancement that has led to larger lesion generation (97).

d) Perfusion Probes

In perfusion electrodes (i.e. XLI-Enhanced and Talon electrodes (AngioDynamics, Queensbury, New York) (83)), saline or hypertonic saline is infused into the target region in order to increase the electrical conductivity of the tissue and enhance the volume of ablation. Interstitial electrolyte perfusion spreads the applied RF current further into the tissue away from the surface of the electrode, allowing a greater amount of RF energy to be delivered to the tissue, while avoiding charring and desiccation at the tip of the electrode (98). Although some have reported increases of up to double the lesion diameter using perfusion electrodes (Goldberg et al., 1996), others have observed unpredictable and irregular shapes of necrosis associated with perfusion RFA (98).

e) Multi-tined Probes

Multi-tined and expandable probes (i.e. the LeVeen device (Boston Scientific, Nantucket, Massachusetts) (83)), use multiple uninsulated prongs deployed from a central cannula, resembling the shape of an umbrella. Each prong creates a separate area of coagulation necrosis, which collectively enhance the ablation zone.
f) **Helical Coil Probes**

A series of large RF helical coil probes developed in early 1980s, took advantage of higher radiofrequencies (i.e. 10-100 MHz) to create axially directed electrical fields and produce transversely uniform deep heating in tissue without excessive heating at the surface layers (99). More recent developments in helical coil probes used the same principles to create small coil applicators, designed for percutaneous soft tissue tumour ablation (100). Specifically, employing a solenoid geometry at high frequencies allows magnetic induction and electrical fields generated inside the coil, and around the conducting wire, which lead to generation of larger, more uniform coagulation volumes in soft tissues (101, 102).

g) **Bimodal electric tissue ablation (BETA)**

Similar to the concept of perfused electrodes, in 2011, Tiong et al., published a dual modality application of RFA which incorporates the process of electrolysis into radiofrequency ablation to increase the size of tissue ablation in soft tissues (103). In particular, they tested the Cool-Tip RF system (Covidien, Boulder, CO, USA) with electrolysis in a pig liver system and found that the dual modality improved the efficacy of ablation by creating larger lesions due to increased tissue hydration which facilitated delivery of the electrical energy and delayed tissue desiccation (i.e. the ablation process continued for longer durations, leading to larger ablated regions) (103). Specifically, this method works because the cathode of a direct current (9 V of DC) circuit attached to the RF electrode, will induce electrochemical reactions that attract water molecules to the tissue surrounding it. This in turn increases the resultant hydration and delayed desiccation (103).

1.5.4 **RFA Applications in Bone**

RFA has been used for treatment of primary bone tumours for a number of years and there are numerous published papers, describing the clinical successes and failures of this technique (104-111). The first report of treatment in the musculoskeletal system was for patients with osteoid osteoma in early 1990s (112). The rationale was simple: conventional surgical excisions have disadvantages including difficulty of locating the lesion intraoperatively, the need for prolonged hospitalization, and potential postoperative complications ranging from unsatisfactory cosmetic outcomes to a fracture (32). However, minimally invasive RFA provided an attractive alternative
for treatment of primary bone tumours, particularly osteoid osteomas (a male-dominant, small bony nidus of less than 2 cm in diameter (106) which accounts for 13.5% of all benign tumours, (32)).

Application of RF in the spine has been historically most common for facet denervation and controlling lower back pain (113-115). More recently, clinicians treating bone metastases have begun to take advantage of this minimally invasive procedure (116-118). The goal of RFA in bone metastasis is pain palliation and slowing the growth of local disease. Therefore, patients who receive RFA may continue to be treated via systemic and conventional methods such as chemotherapy or radiation therapy. Pain palliation is achieved likely due to destruction of the sensory nerve fibers in the periosteum and bone cortex that would otherwise transmit pain. Ablation of tumour burden and debulking of the viable tumour cells may also result in reduction of the lesion volume, decreased stimulation of sensory nerve fibers, inhibition of release of inflammatory modulators and nerve-stimulation cytokines (such as tumour necrosis factor- alpha [TNF-α], interleukins, etc.), and inhibition of osteoclast activity (16, 41, 116, 119, 120).

Although some have identified a lesion located in the spine a contraindication to RFA (32), reports of RFA applied to spinal metastases are growing. Posteraro et al. reported RFA in the metastatic spine of six patients using a multi-array design with nine separate electrodes (R.I.T.A. Medical Systems, Inc., Mountain View, CA, USA) or an internally cooled cluster electrode (Radionics, Burlington, MA, USA). Pain reduction was achieved; however, they experienced residual or recurrent tumour with large metastases in half of the treated patients (119). The first RFA treatment of a human femur’s endomedullar metastasis following primary breast cancer carcinoma was reported in 2008. The RFA device consisted of a multi-tined StarBurst electrode and R.I.T.A Medical generator (R.I.T.A. Medical Systems, Inc., Mountain View, CA, USA) and the tumour was treated in two passes in order to cover the entire range of malignant cells. The result was total necrosis of the tumour that also caused osteolysis of the interior femoral cortex and pain relief and normal daily activities up to three months of follow up (121).

A more recent trend in RFA in bone is local combination therapy using RFA followed by cementoplasty (such as kyphoplasty or vertebroplasty). Lane’s group reported using the combined RFA/cementoplasty approach for painful neoplastic bone metastases with “100%
technical success”, but reported a number of complications, both symptomatic (such as cases of transient thermal sciatic neurapraxia, and transient pain following epidural leaks) and non-symptomatic (including cement emboli to the lung; incidental, non-symptomatic leaks into the needle track, spinal canal, draining veins, and disc spaces; and an intra-articular leak into the hip joint). One reported benefit associated with the dual therapy is greater control over cement distribution that may be particularly valuable in posterior lesions (122).

1.5.5 The impact of RFA on bone and tumour tissue

1.5.5.1 Histologic Evaluation

Several investigators have used histology as an evaluation tool to determine the efficacy of RFA in tissues. It has been suggested that thermal coagulation necrotic effects that result from RFA should take place within seconds to minutes and may have a macroscopic and microscopic presentation of 3-5 distinct zones of necrosis (89). The features of RFA treated lesions stained with H&E have been described as follows: a) a needle track which may demonstrate microcavitation and/or charred area, b) ablated tumour which may show up as intact tumour tissue architecture and cell composition, c) ablated peri-tumoural tissue which may also show up as intact tissue architecture, d) dark rim which demonstrates hemorrhage with typical necrosis, and e) vague outer band, which includes hyperemia, inflammatory infiltration and edema (89). However, RFA coagulated tissue may stain using immunohistochemical assays (89).

VX2 is a widely used preclinical tumour model in studying RFA and other thermal ablation applications. VX2 tumours can also grow within bones, representing a potential preclinical model for studying RFA in tumour involved bone. Authors have reported VX2 tumour development in the rabbit tibia and ilium (15, 123-126). In soft tissues, Ni et al. studied RFA treated VX2 tumour-bearing liver, and reported an inability to distinguish between the ablated and unablated tumour using H&E due to the so-called thermal fixation or ghost effect (89). Similarly, others have reported difficulty identifying viable cells in the ablation area using H&E (89, 127, 128). In these cases, nicotinamide adenine dinucleotide (NADH) staining has shown to sharply circumscribe the edges of ablation (89, 128). Nonetheless, most investigators have reported the use of H&E stains in determining the effects of RFA on VX2 tumour (35, 128-135) and bone (94, 126, 136-141). Unfortunately NADH is not necessarily appropriate for bone as the
decalcification process may impede staining and fresh frozen sections of bone are more difficult to process.

Yamamota et al. evaluated the effect of RFA on healthy femurs of rabbits using H&E and found an intact appearance of osteocytes and the lamellar structures at day 1 post treatment and disappearance of osteocytes, preserved lamellar structure and new proliferation of osteoblasts and osteoclasts at the periphery of ablation zone (136). Lee et al. examined RFA effects on healthy canine femur and described a zonal pattern of necrosis including a central region with severe hemorrhagic congestion and coagulation necrosis surrounded by granulation tissue at four days which became more conspicuous on day 7 (94). Aschoff et al. studied RFA in a healthy porcine femur model at 14 days and reported a central hemorrhage in the tract zone and scattered small foci of hemorrhage in the necrotic marrow farther from the tract. They observed granulation tissue and dilated blood vessels in the outer zones. Many empty lacunae were also reported in the central trabecular bone with viable marrow cells beyond the borders of the lesion (139). More specific stains for bone and/or VX2-bearing bone in RFA studies were not found in the literature.

b) Image-based Evaluation

In line with clinical practice, evaluation of RFA results in bone with MRI has been utilized preclinically in animal studies. Several authors have reported evaluating treatment effects using a number of T1 and T2-based MRI sequences (94, 136, 139-142). While these investigators reported successful bone RFA evaluation using MR imaging based on the hypo-intensive and hyper-intensive imaging signals, their models involved only healthy bone tissue and therefore have not addressed the reported clinical problem of evaluating tumour tissue ablation post treatment in bone (29, 129). Choi et al. reported visualization of VX2 tumour tissue in rabbit tibia using contrast-enhanced MRI but did not treat these tumours. Rather they correlated necrotic and osteolytic features of the tumours seen in imaging to histology (126). RFA ablation of VX2 tumours in soft tissue has been evaluated using MR imaging and demonstrated to have more accurate differentiation between peri-ablational and residual tumour tissue using a blood pool contrast agent SH L 643A (129).
1.5.6 Current RFA Limitations

Limitations in the current state of the art for RFA persist in both bone and soft tissue applications (119). For example, small and incomplete zones of ablation, heat sink susceptibility, needle tract seeding, charring, boiling and other iatrogenic injuries are all common problems. The shortcomings of RFA and its mechanisms of action are well understood in soft tissues. In osseous tissue however, despite the widespread use of radiofrequency for various applications, there is little evidence specifically characterizing the impact of high temperature RF on bone tissue and its cellular components. Rather, studies considering the application of RF devices in bony tissue have focused their reported outcomes mainly on image based tumour tissue ablation and pain scores (41, 107, 108, 110, 116-118).

A major challenge of using RFA to treat large vertebral lesions is the difficulty associated with protecting the spinal cord and the nerves from damage, if heating is deposited very close to the posterior vertebral body wall or in epidural tissue (143), particularly when lesion generation is unpredictable. Limitations such as lesion size, conformality and non-uniform heating can reduce the therapeutic efficacy of traditional RFA in large soft tissue targets (83). In addition, material properties of bone, including its thermal and electrical conductivities differ greatly from those of soft tissues, further complicating RFA application with respect to achieving comprehensive areas of ablation (90).

Current monopolar and expandable RFA array technologies also pose significant challenges in bone that relate to probe deployment and completion of the electrical circuit. The heterogeneous material properties of diseased bone tissue (osteolytic, mixed osteolytic/osteoblastic, and osteoblastic) significantly influence electrical impedance. Finally, in clinical cases where RFA is utilized as an adjunct to vertebral osteoplasty procedures, there is little understanding of the mechanical impact of such a combined therapeutic approach. The few available reports of mechanical investigation of RFA impact on the skeletal system are limited to studies of healthy long bones and joints (136, 144).

RFA outcomes are dependent upon the operator, the imaging tools as well as the device (140). As such, improvement to RF technology is an important step to enhancing treatment results. In bony applications and vertebral ablations, the anatomical architecture and neighbouring critical
structures (such as the spinal cord), require that an ablation zone is generated that is consistent in temperature throughout the region, maintaining 60-100 °C, and can spare the neighbouring critical structures of vertebral bodies. This must be achieved through device design.

In accordance with the aforementioned requirements, there have been two recent developments for improved ablation of spinal metastases using RF. The first is a bipolar cooled radiofrequency probe (BCRF), OsteoCool® (Baylis Medical Company, Mississauga, ON), that takes advantage of a cooling mechanism together with a bipolar circuitry, improving the lesion generation in an osseous environment. The BCRF electrode, was designed and manufactured by Baylis Medical Company, and refined through our collaborative efforts (see co-authorship statements in thesis chapters). It is currently a commercially available product undergoing clinical evaluation in both North America and Europe. The second is a coil probe (RF Bone Coil) designed and developed in house by our team, (University Health Network and Sunnybrook Research Institute). This device uses a non-conventional RF range to create large and consistent lesions by taking advantage of improved power deposition in the tissue through exploitation of both electrical and magnetic fields.

1.5.7 Thesis Rationale

The purpose of this thesis work is to generate and evaluate bone-targeted RF electrode design (BCRF and Bone Coil) and treatment parameters in order to help guide translational development towards the clinic. We determined safety and efficacy in pre-clinical lapine and porcine bone (femur, and spine) models. We determined the best MR imaging protocols for evaluating tumour margins and treatment response and studied the impact of RFA on bone and tumour cells. Finally, we sought to determine the effect of RFA on vertebral strength (leading to stability) and the effects of RFA as a neo-adjuvant to vertebroplasty on spine strength and stability post treatment. Innovation in this thesis considers adding new and important knowledge on the effects of RFA on tumour and bone cells and guiding translational efforts of new bone-targeted RFA probes towards the clinic to treat human disease.

Thesis Hypothesis

We hypothesize that improvements in radiofrequency ablation probes, comprising bipolar cooled designs and high frequency-operated helical coil geometries, can enable safe and successful
tumour ablation within metastatically-involved bones and that will enhance creation of a cavitary defect that when combined with cement augmentation leads to an increase in vertebral stability.

1.5.8 Thesis Outline

This thesis is comprised of four main studies (chapters 2-5), which have been prepared as manuscripts for submission to peer reviewed journals. As well, the initial design and development work and a series of preliminary projects that have enabled these main studies are summarized in the appendices (3.7 and 4.7).

In chapters 2 and 3 we seek to determine the safety and efficacy of the refined RF probe (BCRF) for application in bone using scale-up and diseased small animal preclinical models. In addition, we study the impact of RFA on tumour and bone cells in chapter 3. The preliminary experimentations leading to the animal model, probe and treatment protocol are summarized in the appendix of chapter 3. Note, the engineering development of the BCRF probe was completed at Baylis Medical. In chapter 4, we evaluate our second bone-targeted RF probe (Bone Coil) in a scale-up preclinical animal model and compare the results with that of BCRF (presented in chapter 2). A summary of design, development and validation studies of the Bone Coil electrode utilized in chapter 4 is presented in the appendix of this chapter. In chapter 5, we conduct a comparative study of treatment with the two RFA electrodes, in combination with vertebroplasty, in an ex vivo vertebral metastatic model. In this chapter, we examine the posterior wall movement of the treated level under loading as an indicator of vertebral strength and mechanical stability. The specific objectives, aims and hypotheses for each of these chapters are described below. In chapter 6 a summative discussion of the work and future directions are presented.

Chapter 2: Evaluation of a bipolar cooled radiofrequency device for ablation of bone metastases: preclinical assessment in porcine vertebrae

*Manuscript has been published by The Spine Journal.


Pezeshki PS, Woo J, Akens MK, Davies JE, Gofeld M, Whyne CM, Yee AJ.

Objective: To evaluate the BCRF probe in safely treating healthy porcine vertebrae without neurologic complications and to test the ability of clinical imaging to represent histological
treatment outcomes of RFA in bone.

**Hypothesis:** Single BCRF treatment can create large lesions within healthy vertebrae (approximating human dimensions) without neurologic complications that can be quantified by clinical MR images.

**Aim 1:** To test the ability of BCRF treatment to safely create large lesions (> 2cm (length) x 1cm (diameter)) required for human use in vertebral bone ablation, within a pre-clinical porcine vertebral model.

**Aim 2:** To determine the ability of clinical 3T-MRI to define the region of RFA treatment effect represented histologically, immediately and two weeks following treatment as described in Aim 1.

**Chapter 3: Bone-targeted bipolar cooled radiofrequency ablation in a VX2 rabbit femoral carcinoma model**

*Manuscript has been submitted to Journal of Clinical and Experimental Metastasis.*

**Pezeshki PS, Akens MK, Gofeld M, Woo J, Whyne CM, Yee AJ.**

To determine bone and tumour cell effects, we chose to utilize a diseased femoral model given the lack of a practical large animal vertebral tumour model. From an orthopaedic clinical stability perspective, the vertebrae and proximal weight bearing lower extremity (i.e. femora) are the anatomic regions most relevant when considering the risk of pathologic fracture and functional implications on physical mobility and ambulation.

**Objective:** To evaluate the BCRF probe in the treatment of bone tumours and to compare the effects of RFA treatment on both diseased and healthy bones.

**Hypothesis:** A single treatment with BCRF will effectively and comprehensively ablate large tumours in diseased bone tissue. BCRF will also cause comprehensive death of osteoblasts, osteoclasts, and osteocytes within the RFA treatment regions.

**Aim 1:** To determine the ability of a single RFA treatment with BCRF to ablate tumour within the femur.
Aim 2: To evaluate the effect of BCRF treatment on healthy and tumour involved femoral bone.

Chapter 4: Helical coil electrode radiofrequency ablation designed for application in osteolytic vertebral tumours – initial evaluation in a porcine model

*Manuscript has been submitted to The Spine Journal.

Pezeshki PS, Davidson SRH, Akens MK, Murphy K, McCann C, Sherar M, Whyne CM, Yee AJ.

Objective: To evaluate the ability of the RF Bone Coil system to safely create large lesions within healthy porcine vertebrae.

Hypothesis: Single RF Bone Coil treatment can safely create large lesions within healthy vertebrae (approximating human dimensions) without neurologic complications.

Aim 1: To test the ability of Bone Coil treatment to safely create large lesions required for use in the human spine, within a pre-clinical porcine vertebral model.

Aim 2: To evaluate the radiologic and histologic effects of RF comparing the Bone Coil system to the BCRF probe.

Chapter 5: Evaluating the effect of Radiofrequency Ablation (RFA) alone and in combination with Percutaneous Vertebroplasty (PVP) on the vertebral strength in a simulated osteolytic metastatic model

*Manuscript has been prepared for submission to The European Spine Journal.

Pezeshki PS, Davidson SRH, Murphy K, McCann C, Sherar M, Slodkowska E, Yee AJ, Whyne CM.

Objective: To determine the ability of RFA treatment to improve cement fill and vertebral strength and mechanical stability in cadaveric vertebrae with simulated metastases post PVP. Outcomes will be evaluated comparing RFA using the BCRF and RF Bone Coil systems.

Hypothesis: RFA improves PVP leading to increased vertebral strength and mechanical stability.

Aim 1: To determine the vertebral strength and mechanical stability of the metastatic spine post
RFA treatment (BCRF and RF Bone Coil) in an established cadaveric model of osteolytic vertebral disease.

**Aim 2:** To determine the ability of RFA treatment (BCRF and RF Bone Coil) to reduce tumour volume leading to improved cement fill patterns during PVP, and yield superior post PVP mechanical strength in this model.
1.6 References


Chapter 2
Evaluation of a bipolar cooled radiofrequency device for ablation of bone metastases: preclinical assessment in porcine vertebrae

Abstract

**Background:** Cancer spread to the spine affects bone stability and can lead to pathological fracture and neurological impairment. Radiofrequency Ablation (RFA) has recently gained popularity in treating skeletal tumours. Conventional RFA devices use a monopolar design which limits the ability to comprehensively treating large tumours in bony tissues and may pose risks to adjacent critical normal neurological tissues when applied to vertebrae. New bipolar cooled RFA (BCRF ablation) may generate larger controlled lesions without the same degree of risk to adjacent structures.

**Purpose:** The purpose of this study was to evaluate the feasibility, efficacy and safety of RFA using a new bone-targeted bipolar cooled radiofrequency probe (BCRF) in a porcine vertebral model and to evaluate the ability of magnetic resonance (MR) imaging to represent histologic outcomes of RFA treatment.

**Methods:** RFA was evaluated in three non-contiguous lumbar vertebrae in six Yorkshire pigs (25- 30kg). Using a transpedicular approach for probe placement, two vertebrae received BCRF treatment and one vertebrae served as a sham control. MR imaging and neurological assessments were conducted pre- and post treatment as well as immediately prior to animal sacrifice (n=3 at day 0, n=3 at day 14). MRI ablation zones were compared to hematoxylin and eosin (H&E) stained histological sections. Outcome measures included MR imaging, histology, neurological assessment, and temperature data collected throughout the treatment.

**Results:** With BCRF, large reproducible zones of ablation were achieved, confined within the vertebrae, without damage to adjacent tissues or the spinal cord. All animals demonstrated normal consistent neurological behavior pre- and post treatment. External tissue temperatures around targeted vertebrae were not elevated. MR imaging after 14 days was more effective in demonstrating ablation effects than images on day 0, with radiological findings most apparent on T2-weighted sequences. Histological analysis of samples corresponded well to the zones of ablation observed on MR images (R=0.9, p <0.01).
**Conclusions:** The study demonstrated feasibility, safety, and effectiveness of BCRF ablation of vertebral bone. This motivates ongoing pre-clinical evaluation in diseased models to further explore the potential for its use in clinical treatment of metastatic vertebrae.

*Manuscript has been published by The Spine Journal.*


**Co-authorship Statement**

Padina Pezeshki: Study design, probe refinement, treatment protocol development, surgical assist, MR Imaging acquisition, segmentation and analysis, histology sample preparation and analysis, neurological examination, statistical analysis, writing of manuscript.

Jason Woo (Baylis Medical R&D engineer): probe design and development, treatment protocol development, data acquisition, manuscript editing.

Margarete Akens: Study design, surgical assist, neurological examination, manuscript editing.

John Davies: Histology analysis, manuscript editing.

Michael Gofeld: Grant writing and funding acquisition, manuscript editing.

Cari Whyne: Grant writing and funding acquisition, study design, supervision, manuscript editing.

Albert Yee: Grant writing and funding acquisition, study design, surgeries, supervision, manuscript editing.
2.1 Introduction

Bone metastases are unfortunate and frequent consequences of malignant tumours which affect over 85% of cancer patients at time of death (1). The most common site for skeletal metastasis is the spine (2)(3). Spread within the skeleton can cause considerable morbidity, including bony pain, hypercalcemia, pathological fracture, spinal cord and nerve root compression, and bone marrow aplasia. Collectively these complications are referred to as “skeletal-related events” (SRE) (2, 4, 5). Patients suffering from bone metastasis generally experience a SRE every 3-6 months (6).

Currently, multiple therapeutic approaches, including radiotherapy, surgical resection, and bisphosphonate therapy, are implemented in treatment of skeletal metastases. However, these modalities have limitations that include: tissue tolerance and toxicities associated with radiotherapy; relative patient health and long recovery times for surgical procedures; and, complications such as femoral stress fractures, osteonecrosis of the jaw and renal issues associated with bisphosphonate therapy (1). These constraints render adjunctive local minimally invasive therapies as attractive alternatives. Vertebroplasty and balloon kyphoplasty have gained recent interest as a local minimally invasive spinal therapy to stabilize metastatically involved vertebrae. The ability to cannulate targeted vertebrae using a bone trochar provides an additional opportunity to deliver a bone-targeted RFA probe to biologically ablate tumour tissue prior to the stabilization afforded by the injection of polymethylmethacrylate (PMMA). This multimodality approach is clinically attractive for its potential to both biologically and mechanically treat metastatically involved vertebrae (7).

Radiofrequency ablation (RFA) is a local minimally invasive therapy, most commonly used to treat primary bone (i.e. osteoid osteoma) and soft tissue cancers. The available technology uses a monopolar, single or multiple, probe design whereby the electric current originating from a radiofrequency (RF) generator is emitted through the probe into the neighboring tissues. The ionic current induces frictional heat production, generating a zone of thermal ablation. The electrical circuit is completed via a grounding pad, often placed on the thigh of the patient. This common setup has a major drawback for bone applications, as the osseous tissue has semi-insulative electrical and thermal properties (8). Completing the electrical circuit from the probe to the grounding pad using the common monopolar setup frequently limits the zone of ablation.
Incomplete ablation results in tumour residue leading to higher recurrence rates and associated pain (9). Additionally, extension of the heat towards the grounding pad may result in unwanted damage of neighboring structures. Finally, bone marrow within trabecular structures may act as a heat sink, further impeding ionic flux between contacts which produces a lesion that is smaller than planned. As such, the ability of RFA to treat large structural bone lesions has been limited. Current monopolar RF may also introduce safety concerns when treating metastases adjacent to the spinal cord or nerves (10), as it is possible to pass current through these critical structures and unintentionally injure these tissues. Conventional bipolar devices do not require the use of a grounding pad but rely on electrodes at the end of the two probes that must be meticulously placed adjacent to one another to achieve successful treatment. This requires high levels of operator dexterity, and can lead to treatment effect variability.

To address these limitations for large structural bone applications, a novel bipolar cooled radiofrequency (BCRF) device was developed (OsteoCool®, Baylis Medical Company, Mississauga, ON) which incorporates the active and grounding electrodes on the tip of a single probe, eliminating the need for a grounding pad or a second probe. The bipolar nature of the system was designed to compensate for the thermal and electrical insulating properties of the bone. The internal cooling of the device minimizes tissue desiccation and charring at the probe tip, allowing for formation of larger heat lesions. The purpose of this study was to evaluate the feasibility, efficacy and safety of this new BCRF probe using a porcine vertebral model and also to evaluate the ability of MR imaging to represent histologic outcomes of RFA treatment.

2.2 Materials and Methods

Experimental Design

Ethics approval was obtained for the porcine RFA study from the institutional animal care committee. Six Yorkshire pigs (weighing 25-30 kg) (University of Guelph, Guelph, ON), were randomly divided in two groups. A total of 3 vertebral levels were analyzed from each of 6 pigs. The effect of RFA on the vertebral bone was evaluated at two time points: immediately and 14 days post treatment (Figure 5). In each animal, one lumbar level, i.e. L6, was used as a sham control (RF probe placement only, generator not turned on), while two noncontiguous lumbar levels (i.e. L2 and L4) were treated.
The outcome evaluation parameters were: 1) temperature at the bone surface during RFA, 2) neurological status, and 3) dimensions of the treatment zone quantified with MR imaging and histological analysis of the excised vertebrae.

**Radiofrequency Ablation Device**

The BCRF probe used in this study was OsteoCool® (Baylis Medical Company, Mississauga, ON, Canada). The probe is 17G (1.518 mm outer diameter) and the radiofrequency generator delivered electrical energy at a frequency of 460 kHz, with a maximum hardware output power of 50 W. The system used a default controlled ramp rate of 10 °C/min.

**Anaesthesia**

Both for imaging and the RF procedure, each pig was sedated with an intra-muscular injection of atropine (0.04 mg/kg) and ketamine (15 mg/kg). A 22G catheter was placed in an ear vein for intra-venous access. Each pig was then intubated and maintained anaesthetized with isoflurane in oxygen (1.5-2.5%; 2L) under ventilation. Both prior to and after the procedure, the animals received an intra-muscular injection of 0.05 mg/kg, 0.3mg/ml concentration, of Buprenorphine (Temgesic) (Schering-Plough, NJ, US) or 0.1 mg/kg, 1.5 mg/ml concentration of Meloxicam (Metacam) (Boehringer Ingelheim, CT, US) for pain relief twice daily over 3 days.

**Radiofrequency Ablation Treatment**

Each anaesthetized pig was placed in ventral recumbency position. A stab incision (~1 cm) was created dorsally to targeted vertebrae (localized by fluoroscopy). Under fluoroscopic guidance (BV Pulsera, Philips, Model # MD0709BRM, Saronno, Italy), a 13G (2.41mm outer diameter) bone introducer needle (OsteoCool®Introducer) was positioned via a transpedicular approach into the lumbar vertebral body of interest in order to create a pathway for the probe. After the final positioning inside the vertebral body was confirmed fluoroscopically, the stylet was removed from the introducer cannula and the RF probe inserted in its place. A secondary temperature measurement (non-treatment) probe was positioned dorsally, parallel to the bone needle with the tip resting near the pedicle entry site in order to monitor the peripheral temperature. Once the placements of the two probes were confirmed fluoroscopically, the generator was turned on and the targeted vertebra ablated for 15 min at a set temperature of
65 °C. The heart rate and blood oxygen saturation levels of the animal were monitored during the entire procedure.

**Neurological Examination**

In order to verify that no peripheral neurologic damage resulted from the procedure, a neurological assessment was adapted from Straw et al. to test the responsiveness of the pigs to stimuli as a measure of safety (11). Specifically, porcine motor and sensation responses were evaluated prior to and post treatment for each animal studied.

**MR Imaging**

MR images of the treated levels were obtained post procedure. For the 14-day animal group, a second MR imaging session was also conducted immediately prior to sacrifice. MR imaging was performed on a 3.0T GE Signa scanner (GE Healthcare, Milwaukee, WI), using a clinical spine coil. As per the routine clinical protocol, images were obtained in all 3 planes (sagittal, coronal and transverse) using a 256 x 256 matrix, 16 cm field of view, 3 mm slice thickness, and 0 spacing gap. T1-weighted, TR/TE:557/16.4, and T2-weighted, TR/TE:5513/125.4 (12) scans were performed for each animal. The area and volume of the ablation lesion within bone were calculated based on manually segmented axial images (Amira 5.2, Visage Imaging GmbH, Berlin, Germany). The segmentations were repeated by the same observer on two separate occasions. The segmentation boundaries at the final time points were verified based on visual comparison to 2D histological results and retrospective measurements of the ablation zone. The area and volume of the ablation lesion within bone were calculated based on segmentation of the T2-weighted images as they showed clearer boundaries and more obvious ablation zones than the T1-weighted images. The T1-weighted images were considered in order to help better identify the ablation zone, confirm healthy anatomy, and ensure that neighboring areas were not unintentionally damaged.

**Histology**

The excised vertebral specimens were fixed in 10% buffered formalin and decalcified using 10% formic acid solution. The decalcified lumbar levels were then cut axially through the vertebral body and sectioned at 5mm thickness for staining. Hematoxylin and eosin (H&E) staining was
performed on decalcified axial sections of the vertebrae to qualitatively assess the ablation zone, the effect on bone cells, the marrow cavity within and beyond the ablation zone and potential changes to the adjacent neural or surrounding vertebral tissues. The ablation zone was described based on demarcation and discoloration (i.e. enhanced eosinophilic staining) using H&E. At higher resolutions specifically, cells were evaluated for evidence of coagulative necrosis (i.e. protein denaturation and molten appearance of the cells). Osteocytes were evaluated for viability as judged by presence of intact nuclei at the marginal, central and transitional zones of ablation. Bone marrow was observed for any induced changes when compared to controls. The spinal cord and surrounding connective tissue was compared with the sham control levels to provide comparative evaluation of cell morphologies.

**Comparative analysis**

Pearson’s correlation analysis was used to evaluate the agreement between calculated ablation volumes on day 0 and day 14 MR images. Also, we compared the maximal diameter measured histologically to the maximal diameter measured on the axial MRI slice which most closely resembled the histologic section. The maximal diameters for both MRI and histology were quantified by the same observer on two separate occasions.

### 2.3 Results

**Ablation volume measurements**

The MR images of the RF treated vertebrae corresponded with the macroscopic view post dissection and the histological sections of the samples (Figure 6). Imaging and histological evidence demonstrated a well-confined zone of ablation within the vertebral body that measured up to 2.5cm in length (Figure 6). The average volume calculated based on segmentation of the MR images for all the treated levels was $2.24 \pm 0.90 \text{ cm}^3$. For the sham group, the probe tract was measured at $0.31 \pm 0.06 \text{ cm}^3$. A strong correlation (91%, $p=0.013$) was found between calculated MR ablation volumes on days 0 and 14 on T2-weighted images.
Feasibility and Safety

RFA was successfully applied within the vertebrae of all six pigs. Probe placement was consistent based on intra-procedural fluoroscopy. Neurological examination, demonstrated consistently normal behavior in all pigs pre-treatment, post treatment and 14 days post procedure, indicating that the treatment did not damage any neural tissues. Similarly, MR images demonstrated undisturbed signals in the spinal cord and soft tissues external to the treated vertebral level. Histological analysis of all levels also showed intact and healthy spinal cord for every treated level.

An ancillary thermocouple demonstrated that temperatures in the physiological range were maintained outside the bone with no excessive heating exterior to the vertebral body (Figure 7). An average maximum temperature of 65.0 °C (range 64.89-65.05 °C) was experienced by the target tissue at the tip of the probe for the treatment duration of 15 min (less the initial 2-3 min ramping period) vs. an average of 36.1 °C (range: 32-40 °C) measured by the secondary thermocouple on the outside of the vertebrae. Average tissue impedance during the procedure was measured to be 270 ± 25 Ω. The generator’s average power output, voltage and current values post ramping were 5.3 ± 1.0 W, 42.5 ± 3.5 V, and 163 ± 23 mA, respectively.

MR Imaging

RF lesions were most apparent on the T2-weighted sequences at all time points. In the experimental group, T2-weighted images showed a combination of hypointensive and hyperintensive regions, often demonstrating a hyperintensive peripheral rim. In general, the axial T2-weighted images demonstrated more morphological details when compared to T1-weighted images. Lesion shapes were consistent on 0 and 14-day T2-weighted images. However, much clearer delineation of lesions was observed on 14-day images (Figure 8). Although the probe insertion track was readily visible on the T1-weighted images, the zone of ablation and treatment effects were not clear for most samples at day 0 imaging. Nevertheless, the coronal T1-weighted images after 14 days were helpful in determining the ablation regions demonstrating a hyperintensive center surrounded by a hypointensive rim (Figure 9, Figure 10). The MR images of the sham control group (probe placement only, no RF delivery) served as a baseline and confirmed that no artifacts were present in the imaging. The sham probe track was visible in
images taken immediately after the procedure on day 0, but was not as easily detectable at day 14 (Figure 11).

*Histology*

Gross histological analysis of the survival samples demonstrated distinct regions of effect that corresponded with bright and dark signals of MR images (Figure 6). Low magnification analysis of the treated samples highlighted the ablated area with clear demarcation (exaggerated eosinophilic staining) as compared to the untreated regions, particularly at day 14 (Figure 12). The probe track was filled with blood pools and hemorrhage in 0-day samples and fluid and fibrous tissue in 14-day samples (Figure 12) both in treated and sham groups. Comparison of histologic and MR measures of lesion diameter at days 0 and 14 yielded a strong correlation (R=0.9, p<0.01). Further Bland-Altman analysis of these results demonstrated an average bias toward a greater diameter size of 1.5 mm in the MR images as compared to the histologic analysis (i.e. average diameter size measured using MRI and histology were 11.4 mm and 9.9 mm respectively).

Examination of the 14-day samples at higher magnification, revealed a region of effect that impacted the bone marrow’s hematopoietic cells. In most cases, the marrow was replaced with fibrous tissue, inflammatory cells and new vessels. Erythrocytes and white blood cells were abundant in the treated areas. The stromal structures of the vertebrae (including the bone and fat cells) appeared less altered morphologically in comparison to the hematopoietic cells.

In the center of the ablation region and along the probe insertion path, both live and dead osteocytes were found. Larger concentrations of dead osteocytes were found in the necrotic region constituting the central two-thirds of the treatment area. More moderate necrosis was observed at the proximal and distal ends of the treatment area, along the probe track. Patches of rapidly forming bone containing live osteocytes were visible throughout the treatment area; live osteocytes and osteoblasts were more abundant at the periphery of the ablation area. Osteoclasts were more abundant at the distal and proximal ends of the treatment region as compared to the central zones, but much fewer than osteoblasts in the area. Fibrous-like tissue and inflammatory cellular components were plentiful in all regions. Nonetheless, necrosis from the treatment
procedure remained in some areas. Minor mechanical damage was induced in the sham vertebrae but overall the bone cells and marrow content were intact.

2.4 Discussion

In this study we have demonstrated the feasibility, efficacy and safety of an improved RFA probe designed for use in osseous tissue. The BCRF probe was able to create large \((2.24 \pm 0.90 \text{ cm}^3)\) repeatable lesions within porcine vertebrae through the generation of internal temperatures above 65°C, while maintaining physiologically normal temperatures external to bone. In order to avoid an additional open surgical laminotomy, we opted to place a secondary temperature probe near the probe’s entry site and the nerve roots, as opposed to the spinal canal. Prior \textit{ex vivo} validation experimentations demonstrated a thermal profile confined to bone. This \textit{in vivo} study further demonstrated that there were no negative effects of RFA on neurological outcomes in treated animals with the application of a BCRF probe inserted through percutaneous bone trochar needles used clinically. Well-defined ablative zones were characterized post-operatively by MR imaging and correlated well to the region of effect determined histologically.

As minimally invasive therapies become increasingly prevalent, the need for designing tissue-specific therapeutic strategies becomes more apparent. RFA probes have been developed and utilized for numerous soft tissue applications (liver and renal tumours, cardiac and spinal denervations) (13)(14)(15), however, these same devices are not ideal for treating skeletal tissue. A challenge associated with RFA in bone is generating comprehensive lesions in view of the relatively low thermal (0.15 to 0.3W/m°C) and electrical conductive (0.01s/cm) properties of bone (8). Current available technology (designed for soft tissue) has been shown to fall short in comprehensively treating bone lesions. In a retrospective study of RFA for the treatment of bone metastasis, Kashima et al. found only 30% of 40 patients had complete tumour ablation and one patient suffered from transient nerve injury (9). Similar complications and limitations have been reported by others evaluating RFA in bone (16)(17)(18). In our experiments, we demonstrated that the BCRF OsteoCool® device placed via the intrapedicular approach is safe to use in the porcine lumbar vertebral body (no neurologic complications) and may create a predictable lesion size contained within the vertebra. No technical difficulties were experienced with respect to the procedural approach or with the device and technology. An advantage by design, is that the
device can be inserted directly through conventional percutaneous delivery bone trochar needles clinically used for vertebral cannulation in vertebroplasty and balloon kyphoplasty.

It has been alluded to in the literature that heat generated from the exothermic vertebroplasty or balloon kyphoplasty procedures may lead to pain control by means of thermal deafferentation (19, 20). As such, RFA may also possess thermal deafferentation effects that are desirable in diseased bone from a pain perspective. Cementoplasty procedures such as kypho/vertebroplasty involve deposition of known volumes of cement inside the centrum of the vertebral body (7, 21, 22). The injection of cement (or balloon inflation) requires physical displacement of existing tissue within the vertebra (i.e. marrow). In the metastatic spine, cement injection can cause physical displacement of tumour tissue/cells, potentially leading to local disease advancement through physical holes in the vertebral cortex or systemically via the vascular network. Radiofrequency ablation prior to cement injection can serve to both destroy live tumour cells and may reduce the volume of tumour tissue by thermal effects. In a pre-clinical study, Ahn et al. demonstrated the ability of laser-induced thermal therapy to reduce tumour volume leading to enhanced cement fill that improved vertebral strength and mechanical stability and lessened cement extravasation (21). Recognizing that there is always a risk of tumour extravasation with cement augmentation procedures, it would be preferable that these tumour cells be non-viable.

Use of conventional monopolar RF technology in similar sized healthy porcine vertebrae, has been shown by other investigators to create lesions which conduct beyond the vertebral cortex injuring surrounding tissue (12). This is of concern from a clinical perspective in treating vertebrae due to the proximity of adjacent neural tissues. With the BCRF probe using the parameters described, extra-cortical temperature measurements of ~36°C confirmed the confinement of the ablation zone within the vertebrae. Observed effects of a well-confined ablation zone may be explained, in part, by placement of the electrical return electrode on the active tip of the probe, better controlling lesion volume with less dependency on the conductivity of the adjacent tissue. Similarly, since the BCRF OsteoCool® probe is temperature-driven, the RF current and power output are adjusted according to the tissue temperature as opposed to tissue impedance (as is the case with most conventional devices). Use of a transpedicular or parapedicular approach to access the vertebral body leads to paramedian RF probe tip placement in the axial plane (as opposed to a probe tip located directly in the centre of the vertebral body).
An ellipsoidal geometry in the axial plane conforming to vertebral anatomy may afford greater coverage of that side of the vertebral body when compared to a spherical geometry that may place the neural elements of the spinal canal at greater risk. If required, depending on tumour location, a bilateral ablation could facilitate more comprehensive treatment of the body of the vertebrae where most metastases are clinically located.

Medical imaging is an important clinical tool to assess treatment effects and efficacy, yet critical image based evaluation of specific bone RFA effects has been limited (12)(23)(24)(25). In this study we observed that RF ablation effects can be detected using MR imaging immediately post procedure (day 0), consistent with findings of Nour et al. (12). However in contrast with Noor et al., the lesion size in this study, as determined by T2-weighted MRI, was maintained over time. The lesion visibility was enhanced at day 14 (particularly in the T2-weighted images), reducing any uncertainties with respect to the ablation zone. The improved ability to delineate the lesion size may reflect the delay (5-7 days) required for necrosis to become apparent following focal heat treatment (26). MR imaging was found to reliably detect a treatment zone consistent with histological findings, particularly a few days post procedure once necrotic effects have settled. While in this healthy bone model we did not apply contrast agents, their use may be beneficial in vertebrae with metastatic involvement to distinguish the ablation zone from any remaining viable tumour tissue. In fact, Aschoff et al. concluded that contrast-enhanced T1-weighted images were the second-most useful modality, following T2-weighted images, for detecting bone lesions in their experimental femurs (23). Overall, these findings motivate future work automating ablation volume calculation using MR images, to remove the potential for any operator dependant error.

Live and dead bone cells were found throughout the ablation zone at day 14. The live cells may have remained intact during the RFA procedure or may have been newly regenerated over the course of 14 days. The histological findings in this study showed that RFA treatment had an even more severe effect on the bone marrow, which was later followed by a physiological repair process. Although marrow was necrotized by RFA, its ability to heal and eventually repopulate normal hematopoietic contents may be preserved. Similar to our observations, Tang et al. suggested that in general, pathological necrotic marrow can heal, possibly leaving small fibrous scar tissues behind (although potential bone marrow fibrosis may be a predisposing factor for idiopathic myelofibrosis) (27). Moreover, unlike avascular necrosis, bone marrow necrosis has
been reported not to progress to vertebral collapse (27). As such, the marrow necrosis induced by RFA may not yield a negative effect on vertebral stability and may stimulate healing and tissue regeneration within the marrow space. Bone tissue has unique thermal and electrical conductive properties that result in a reduction of conduction of the RFA AC current as compared to soft tissue. As such, in the evaluation of technology aimed at treating bone disease, it is important to evaluate the ability to ablate soft tissues that reside within a bony network. The bony structure of the vertebrae in all samples remained grossly, radiologically, and histologically intact post RFA. Imaging and histological observations did not suggest any cortical thinning in our studies. This finding was consistent with results of Cantwell et al. who examined cortical thinning after RFA of porcine long bones and found no cortical bone thinning (with a single exception in the humeral group) (24).

While the clinical indication for the BCRF probe is treatment of spinal metastases, our device was tested in non-pathologic vertebrae due to a paucity of available and practical large animal models of vertebral metastases. The higher bone density in porcine as compared to human vertebrae limits the size of ablation achievable with this device. Further, metastatic disease in the human spine may result in lesions that breach the cortical shell; safety of BCRF utilization in this scenario remains to be evaluated. The study was also limited in that direct measurements of spinal cord heating were not performed. Yet, placement of the temperature probe near the foramen demonstrated physiologic temperatures and no neurologic sequelae were observed. Heating of bone to high temperatures can reduce bone toughness and elasticity (28), however the temperatures maintained during RFA reduce the potential for a heat induced negative impact on the vertebral mechanical properties. However, mechanical testing was not conducted on the vertebrae post treatment to determine the impact of BCRF treatment on bone strength.

2.5 Conclusions

In conclusion, this study confirmed the feasibility of the BCRF OsteoCool® probe in ablating large vertebral volumes in clinically acceptable RFA timeframes. Safety was demonstrated through neurologic evaluation, bone surface thermal measurements, MR imaging, and histologic evaluation. Moreover, strong correlations were observed between quantitatively assessed MRI ablation zones as compared to those determined histologically. Future evaluation of the BCRF
OsteoCool® probe in pathologic pre-clinical models will further guide the application of the device and evaluate its effectiveness in treating metastatic disease in bone.

Figure 5. Experimental design flow chart.
Figure 6. L4 porcine vertebra treated with BCRF. (a) T2-weighted axial MRI 14 days post-procedure. (b) macroscopic view of the sample. (c) H&E stain of the same sample.

Figure 7. A representative graph of the generator output during the ablation procedure, demonstrating typical power, impedance, voltage and temperature values of the tissue at the probe tip as well as external temperature measurement from a secondary thermal probe. Cooled Temp A represents our set temperature of 65 °C and Cooled Temp B represents the probe tip temperature collected throughout the treatment.
**Figure 8.** T2-weighted MR imaging post RF treatment at (a) day 0 and (b) day 14. The region of effect is more clearly defined at day 14.

**Figure 9.** MR images of an RF treated L4 vertebra showing the large region of effect that is circumscribed within the vertebra. (a) T2-weighted sagittal view and (b) T1-weighted coronal view.
Figure 10. T1-weighted coronal images at (a) day 0 and (b) day 14 post RFA treatment. These images demonstrate the improved effectiveness of MRI in determining treatment outcomes 14 days post-procedure. Note that T1-weighted imaging on day 0 displays only the probe entry route (arrows), identical in the sham control and experimental levels. Only after 14 days does the treatment effect become visible.
**Figure 11.** T2-weighted MR imaging of the sham control (probe placement only) samples, axial view at (a) 0 days post procedure, demonstrating probe tract, and (b) 14 days post procedure, probe tract is less evident.

**Figure 12.** (a) Low power H&E section immediately post treatment (day 0) showing hemorrhage in the probe track and little to no evidence of marrow damage. (b) Low power H&E section 14 days post treatment, clearly demonstrating the region of effect through discoloration and cytoplasmic eosin staining. (c) High power magnification 14 days post treatment: a number of dead osteocytes (● identified by their shrunken nuclei or empty lacunae), active osteoblasts laying down new bone (●), fibrous tissue (▲) and numerous new vessels (▲ demonstrated of angiogenesis) are visible.
2.6 References


Chapter 3
Bone-targeted bipolar cooled radiofrequency ablation in a VX2 rabbit femoral carcinoma model

Abstract

PURPOSE: To determine the effect of bipolar cooled radiofrequency ablation (BCRF) on bone and tumour cells in a diseased lapine model, and evaluate MR imaging in reflecting histological ablation in bone.

MATERIALS AND METHODS: Under institutional approval, twelve New Zealand White rabbits received an injection of VX2 carcinoma cells into one femur (day 0). Rabbit femora were block-randomized into four experimental groups: tumour-bearing radiofrequency ablation (RFA) treated, healthy bone RFA treated, tumour-bearing shams and healthy bone shams (n=6 per group). Fifteen minutes of thermally regulated (65 °C) BCRF was applied at day 14. Pre- and post treatment MR imaging was performed and repeated at day 28 prior to euthanasia. Histologic evaluation (H&E, AE1/AE3, TRAP and TUNEL) was performed on all samples.

RESULTS: Large volumes (12.9 ± 5.5cm\(^3\)) of thermal ablation were achieved. An eight-fold reduction in tumour growth was observed in RFA treated animals when compared to tumour-bearing sham controls (p<0.001). Therapeutic effects were best imaged using MR contrast-enhanced SPGR sequences. Osteoclasts and osteoblasts were observed to be sensitive to RFA. A small number of tumour cells and osteocytes within RFA treated regions appeared viable post treatment. New bone formation was stimulated in the periphery of the targeted RFA treatment zone.

CONCLUSIONS: Structurally large VX2 tumour volumes within bone were successfully ablated, stimulating new bone formation in the treatment periphery. The ablation zone was best distinguished using contrast enhanced MR imaging, although histologically, viable appearing osteocytes and tumour cells were observed in some treated regions. Further study is needed to understand the cellular mechanisms and guide RFA therapeutic optimization within tumour involved skeletal tissue.
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Co-authorship Statement

Padina Pezeshki: Study design, probe refinement, treatment protocol development, surgical assist, MR Imaging acquisition, segmentation and analysis, histology sample preparation and analysis, post-op assessment, statistical analysis, writing of manuscript.

Margarete Akens: Study design, surgical assist, histology sample preparation and analysis, manuscript editing.

Michael Gofeld: Grant writing and funding acquisition, manuscript editing.

Jason Woo: Baylis Medical R&D engineer, probe design and development, treatment protocol development, data acquisition, manuscript editing.

Cari Whyne: Grant writing and funding acquisition, study design, supervision, manuscript editing.

Albert Yee: Grant writing and funding acquisition, study design, surgeries, supervision, manuscript editing.
3.1 Introduction

Advances in bone-targeted radiofrequency ablation (RFA) aim to improve quality of life for many patients who suffer from skeletal metastases. Conventional therapy for bone metastases includes systemic bisphosphonates (1), radiation therapy and in carefully and appropriately selected patients, interventional radiology and/or surgical approaches. Radiofrequency ablation (RFA) has been used clinically to treat painful non-mechanically significant primary and secondary skeletal lesions (2-7). However, RFA treatment of structurally large bone lesions remains a challenge (8).

RFA functions by directing alternating electrical current to locally excite ionic cellular components, relying on successful heat conduction and completion of an electric circuit. The application of conventional RFA has been successful in treating soft tissues and structurally confined, small primary bone tumours (i.e. osteoid osteomas) (9)-(10). However, there are limitations to conventional electrode applicators, historically designed for soft tissues when considering their potential application to larger structural diseased human bone. Existing challenges include therapeutically small zones of ablation, inconsistent regions of thermal effects, tissue carbonization and desiccation (11). A challenge for RFA application in larger osseous lesions representative of skeletal metastasis is completing an electrical circuit in bone and achieving sufficient conduction, which depends on the thermal and electrical properties of bone (12).

There is limited scientific literature concerning evaluation of RFA for bone tumours, in particular for structurally large bone lesions (13-15). Advancements in RF electrode applicator design have enabled the generation of structurally large ablation volumes within skeletal tissue, however the impact of RFA on large areas of tumour involved bone have not been well described. This study aims to determine the effect of bipolar cooled radiofrequency ablation (BCRF) on bone and tumour cells in a diseased lapine model, and to compare MR imaging effects in reflecting histological ablation within the diseased skeleton.
3.2 Materials and Methods

Experimental Design

Institutional animal care ethics approval was obtained for the study. Twelve New Zealand White (NZW) rabbits underwent an intramedullary injection of VX2 tumour cells into one randomly selected femur. Bone tumours developed over 14 days following cell inoculation. A thirteenth animal (also injected with VX2 tumour cells) was sacrificed as a control for histology on day 14 and MR scanning to select the sequences for bone tumour radiologic evaluation that best highlight tumour burden.

In the remaining twelve animals, a block randomization protocol assigned animals to one of 4 experimental groups: tumour-bearing RFA treated, healthy RFA treated, tumour-bearing sham and healthy sham groups (n=6 per group). RFA or sham treatments were applied on day 14. Treatment effects were evaluated by MRI performed on day 28. Animals were euthanized and bone tissues harvested (Figure 13). This time point following RFA was selected based on our preliminary work and a literature review of published VX2 studies in rabbit long bones (16-20).

Femoral VX2 tumour cell injection

Anesthesia in each rabbit was induced by intra-muscular injection of ketamine (15mg/kg) and xylazine (2mg/kg) with maintenance inhalation anesthesia (2-3% isoflurane in oxygen (2L/min)). A small incision (2 cm) was made at the distal end of the femur through which a small hole was drilled (1.2 mm diameter) for tumour cell injection (200µl of VX2 cell suspension (~5x10³/µl) using a 25 gauge needle). The drill hole was placed at the distal femoral intercondylar notch. Following cell injection, the drill hole was sealed with bone wax and the incision suture closed.

Bone-targeted radiofrequency ablation

A bipolar cooled radiofrequency (BCRF) applicator (OsteoCool®, Baylis Medical Company, Mississauga, ON, Canada) was used to administer the procedure. The BCRF system included a 17G (1.518 mm outer diameter) electrode applicator and a RF generator that delivered electrical energy at a frequency of 460 kHz (maximum hardware output power of 50 W). The system used a default controlled ramp rate of 10 °C/min such that a final temperature of 65 °C was reached.
after approximately 3.5 minutes. A thermocouple at the tip of the applicator continuously measured the temperature throughout the procedure. This temperature feedback maintained the applicator tip at the set temperature and prevented charring. On the day of RFA therapy (14 days after cell injection), each rabbit was anesthetized, and the intercondylar notch drill hole accessed to position the RFA applicator into the femoral medullary cavity. In the tumour group, pre-treatment MRI helped locate the tumour within the femur and measurements based on the images, guided subsequent electrode applicator placement. Under fluoroscopy (BV Pulsera, Philips, Model # MD0709BRM, Sarronno, Italy), the electrode applicator was positioned into the tumour residing within the intra-medullary cavity, with the radiopaque band (located at the proximal end of the active tip) of the BCRF electrode applicator positioned approximately at the distal edge of the tumour. To monitor heat effects outside the bone, a second temperature sensor probe (22G) was placed exterior to the surface of the femur and near the ablation electrode applicator tip (Figure 14). The treatment duration was 15 minutes (including ramp up time) with a 65 °C set temperature. For sham control samples, the electrode applicator was placed and no electrical current was applied. The heart rate and oxygen levels of each rabbit were monitored during the procedure. After the tumour cell injection and the RFA procedure, the animals were observed daily to assess their overall wellbeing and their level of lower extremity load bearing as a qualitative measure of treatment safety.

*Magnetic Resonance Imaging*

Imaging was performed on rabbit femora under anesthesia prior to and immediately following RFA treatment (day 14) as well as immediately prior to animal sacrifice (day 28) on a 3.0T GE scanner (GE Healthcare, Milwaukee, WI) using a 5” surface coil. Images were acquired using standard 3D-SPGR (SPOiled Gradient Recalled) and 3D-FIESTA (Fast Imaging Employing Steady State Acquisition) sequences. A typical field of view of 16 cm (matrix = 256 x 256), slice thickness of 3mm, TR/TE 8.3/3.1 ms and flip angle of 55 was used for the FIESTA sequences. A typical field of view of 16 cm (matrix = 512x512), slice thickness of 3mm, TR/TE 4.4/1.6 ms and flip angle of 30 was used for the SPGR sequences. Gd-DTPA contrast agent (0.1mmol/kg) was injected intravenously and post contrast images were acquired. All images were analyzed to quantify surface area and volume measurements of the tumour and to assess the ablation zone (including the peripheral rim) by manual segmentation of the region of effect (Amira 5.2
software, Visage Imaging GmbH, Germany). Results were then compared to histology images (described below), to confirm the zone of ablation and qualitatively assess the ease of detection of the ablated regions using MRI.

**Histology**

The femora containing tumour and surrounding thigh soft tissues were harvested post sacrifice. Specimens were fixed in 10% neutral buffered formalin. To facilitate EDTA decalcification(21), the femora were cut transversely at the proximal ends (i.e. distant to tumour and therapy region). Hematoxylin and eosin (H&E) staining was used to assess the osseous ablation zone. TRAP staining was used to identify osteoclast activity in each of the diseased and healthy samples. TUNEL staining was used to identify apoptotic cells in bone. Cell death was determined by counting the number of cells containing nuclei with apoptotic morphology that were also stained dark brown with 3,3’-Diaminobenzidine (DAB). A positive pixel count algorithm was used. Finally, AE1/AE3 cytokeratin staining of cells of epithelial origin was used to determine VX2 tumour cell necrosis. Cells with melted, indistinct membrane and coagulated cytoplasm and nuclei were counted as treated and dead, while intact, plump cells with clear healthy morphology were considered unaffected.

**Study Design and Analysis**

Sample size was determined for a parallel study, with standard deviation and difference-in-means input values determined based on the size of ablation zones (i.e. volume of an ovoid) from our pilot experiments. A bonferroni correction was performed to adjust for the type 1 error associated with multiple comparisons in the study. Statistical analysis of the two treatment parallel-design study was conducted using a Student’s t-test to compare pairs of independent groups by means of PASW software (Version 18.0, Chicago, IL, USA).

3.3 Results

**VX2 tumour growth and application of RFA**

VX2 tumours were generated in all injected rabbit femora. Manual segmentation of the MR images revealed generated femoral tumours ranging from 0.9 to 1.9 cm$^3$ in volume (average 1.4
± 0.5 cm³) at day 14 (Table 2). RFA treatment was technically successful without any device-related issues or disruptions. The RF generator was stable through the procedure delivering a consistent energy output of 5-10 W to treated femora over the prescribed 15 minutes.

**Clinical and imaged based RFA effects**

*a) Ablation size*

The MR images of the RFA treated femora consistently revealed zones of ablation that affected intramedullary and cortical bone regions as well as the surrounding soft tissues of the thigh. Ablation volumes of tumour bearing and healthy bones based on all MR sequences confirmed an average of 12.9 ± 5.5 cm³ of treated volume (with an average length and diameter of 3.1 ± 0.3 and 1.5 ± 0.3 cm respectively) including both bone and adjacent soft tissues (Table 1). [Note: the size of ablation was relatively large compared to the rabbit anatomy as the device used was designed for human application and anticipated human volumes].

*b) Effect of treatment on the tumour in bone*

The large zone of ablation created did not always correspond with the tumour distribution along the femur; viable tumour cells remained outside the treatment volume in all samples (Figure 15). In two of six RFA treated tumour bearing animals, a small number of viable tumour cells were observed histologically (H&E and sAE1/AE3) within the RFA treated region. The size of tumour was reduced in RFA treated animals with an eight-fold reduction in viable tumour volume at day 28 compared to the untreated tumour bearing RFA sham group (p<0.001) (Table 2 and Figure 16). Tumour growth in the RFA untreated sham group extended beyond the cortex at the distal femoral end, forming new masses typically in the surrounding soft tissues.

*c) Clinical outcomes*

The rabbits were clinically assessed, by a trained veterinary technician, before and after tumour cell injection as well as post RFA treatment. Special attention was paid to weight bearing of the injected/treated leg. All rabbits tolerated the tumour injection well and did not show any signs of lameness. After the RFA treatment, the rabbits showed low levels of pain (decreased appetite and movement), which was successfully treated with subcutaneous injection of buprenorphine over 2
days. No significant weight loss was observed in any of the animals. Only one rabbit showed additional signs of a slight hind limb lameness, which may have been due to the treatment damaging femoral nerve branches. This was diagnosed after a neurological examination of the rabbit (22). The mild degree of lameness did not interfere with the overall wellbeing of the rabbit and therefore it was not excluded from the study.

d) MRI

Tumour tissue was detectable prior to RF ablation using all four imaging sequences. The tumour features were most easily discernible using Gd-enhanced SPGR sequences. Post ablation however, it was more difficult to distinguish the effects of ablation and necrosis on bone and tumour from residual or live tumour using MR imaging alone. As such live tumour was selected on the images after comparison with histological evidence. The ablation zone was best distinguished from remaining tumour and healthy tissues using contrast enhanced MR imaging (Gd-SPGR and Gd-FIESTA). Since MRI does not provide any information regarding mineralized tissues, the cortical femoral shell appeared intact in these images, without any signal change among groups (Figure 16). A significantly higher volume of tumour was detected in the sham group compared to the treated group (average of 2.58 ± 0.00 vs. 0.35 ± 0.09 cm³ respectively, p<0.001). The ablation volume was 1000x greater than the volume of injury induced by electrode applicator placement in the sham group (p<0.001).

Histologic effects of RFA on bone

H&E staining demonstrated enhancement in the treatment region of the experimental group (Figure 17). Coagulative necrosis was evident in the medullary cavity of these treated regions surrounded by areas of fibrous tissue formation (healthy group) and VX2 cells (tumour-bearing group) at the periphery of the RFA treatment zone. TUNEL staining confirmed RFA apoptotic effects on bone cells in the cortex as well as along the periosteum and endosteum of the cortex.

As expected, tumour-bearing femora exhibited a significantly elevated level of osteoclastic activity when compared to the healthy control femora (Figure 18). Osteoclasts were observed in high concentrations directly adjacent to tumour tissue. In addition to an overall increased number of large (≥3 nuclei, p<0.001) and small (<3 nuclei, p<0.005) osteoclasts, tumour-bearing femora
exhibited a higher percentage of large to small osteoclasts when compared to healthy control femora. TRAP staining demonstrated a significant reduction in osteoclast counts (both large and small) with RFA treatment compared to the respective untreated control groups.

TUNEL staining showed a high number of apoptotic bone-lining osteoblasts in the RFA treated femora (both tumour bearing and healthy groups). Unstained cells demonstrating necrotic morphology were characterized as effectively treated. In every RFA treated sample, the bone cortex along the periphery of the RFA treated region demonstrated an increased number of osteoblasts with new subcortical trabecular bone formation. The visible new bone formation seen only in the RFA treated groups averaged 4.04 ± 3.40 mm² for the VX2-bearing femora and 5.78 ± 0.64 mm² for the healthy femora as measured on H&E stained sections (Figure 17).

While H&E staining of specimens demonstrated some empty lacuna, a large number of healthy appearing osteocytes were visible post RFA treatment in both healthy and tumour-bearing groups. However, we noted that subsequent TUNEL staining demonstrated DNA fragmentation in many of these cells, indicating a RFA-driven treatment effect. We also observed intact osteocytes within the RFA treated region of 5 of 12 animals. Increased TUNEL staining in the cortex was observed in 3 of 12 animals where the electrode applicator was fluoroscopically observed at the time of procedure to be touching the cortex.

### 3.4 Discussion

There is limited scientific literature concerning evaluation of RFA for bone tumours, in particular for structurally large bone lesions (13-15, 23). The available literature evaluates the efficacy of RFA in healthy bone as a model for metastases. In our study, RFA significantly reduced the number of viable tumour cells and suppressed growth of tumours when compared to non-RFA treated tumour controls (eight-fold reduction by tumour volume, p<0.001). However, viable tumour cells were still observed in the RFA zone in one third of the RFA treated tumour bearing rabbits. Ablative effects were also observed on bone cells (osteocytes, osteoblasts, osteoclasts) and on periosteum and endosteum, within the treatment volumes as well as bone reactive changes in the skeletal periphery of the ablation zone.
Following RFA, a surprisingly large number of healthy appearing osteocytes were visible in both healthy and tumour-bearing cortical bone, although subsequent TUNEL staining demonstrated DNA fragmentation in many of these cells, indicating a RFA-driven treatment effect. This may be due to a temporal affect or the inherent tissue properties of bone, as described further below.

Wang et al. suggested that full RFA effects (in soft tissues) cannot be visualized using H&E in time points earlier than four weeks (24). Similar to the current study, Yamamoto et al. found that RFA did induce bone cell death. They found at day 7 osteocytes shrank and periosteum disappeared in the centre of the cortical bone ablation area, with osteocytes no longer evident by day 30. This suggests that the changes seen in the osteocytes in the current study at day 14 post treatment may represent a treatment effect not fully realized from a temporal perspective. Similar to the increased number of osteoblasts with new subcortical trabecular bone formation found at the bone cortex along the periphery of the RFA treated region in our study, Yamamoto et al. also noted a proliferation of bone cells and new bone formation at the periphery of the RF ablation zone (25).

In bone, while Lundsgok reported instant necrosis of osteocytes after 30 seconds of exposure to temperatures of 50 °C (26), Tellotson et al. (15), experimenting with different monopolar RFA electrode applicators in healthy dog femora, found complete necrosis of bone marrow yet visible intact osteocytes and variable osteonecrosis of the cortical bone in the treatment zone. These findings were noted regardless of electrode applicator size evaluated or duration of heating. The conventional monopolar electrode applicators used in Tellotson’s work however, yielded only small zones of ablation (spherical, typically 1 cm in diameter). In an ex vivo RFA study of bovine bone, Rachbauer et al. observed that while the average temperature of the bone marrow 5 mm distant to the RF electrode applicator tip was 90.9 ± 26.0 °C, the average temperature recorded at the cortex 5 mm away was only 64.3 ± 13.7 °C and at 10 mm this thermal effect dropped to 41.7 ± 2.2 °C (13). It may be possible that in vivo, the potential heat sinking effect of perfused blood vessels could lead to the generation of lower effective RFA temperatures, possibly leading to sparing of osteocytes.

The observed patchy necrosis of the osteocytes may also stem from the relative resistance of cortical bone and mineralized tissue to RFA effects (which may be ten to a hundred times greater
when compared to solid organ tissues such as liver or bone marrow (13)). This may explain the observed soft tissue necrosis adjacent to the cortical shell despite the seemingly healthy osteocytes within the cortical shell in our study. Thermal effects may be lessened by heat sink effects of the spinal fluid or nearby vasculature. An interesting observation of our study was the significant ablation effects of RFA on cortical bone in those femora that were fluoroscopically visualized during experimentation where the electrode applicator tip contacted the cortex. As such, intramedullary placement without cortical contact is desired to limit unnecessary cortical necrosis. It remains important therefore to optimize treatment parameters and electrode applicator positioning so as to target diseased tissue and preserve adjacent normal tissues.

Recent work in healthy rabbit femurs treated with RF ablation demonstrated similar high intensity rings on T2-weighted and STIR MR images at early time points as seen in healthy porcine vertebrae treated with BCRF and other reports in the literature (14, 25, 27, 28). However, the high intensity rims decreased in size over time. These high intensity rims on T2-weighted in the outermost zones have been reported to correspond to granulation tissue, with hypo- and iso-intense areas in the inner zone corresponding to hemorrhagic congestion and coagulation necrosis (14). No changes in the mean fracture loads of the RFA treated femora were found, suggesting that the heating did not impair bone stability up to 60 days post treatment (25).

Our observations of an increased number and size of the osteoclasts in the sham tumour bearing group (compared to the RFA treated tumour bearing group) are consistent with reported literature. Virmani et al. has highlighted the hypoxic nature of VX2 tumours (29) and Bozec et al. demonstrated that hypoxia induces an increase in the size of osteoclasts (30). Since tumour osteolysis associated with metastasis is more aggressive at an early phase (when the tumour cells are establishing themselves and bone resorption proceeds rapidly, recruiting higher number of osteoclasts) (31), application of RFA for tumour control affecting osteoclastic activity may be desirable when treating during this earlier phase. In addition, an early planned stage of tumour ablation may result in a more homogenous lesion (since tumour necrosis and central tumour cavitation, a finding associated with advanced lesions, has not yet occurred).

Histological analysis confirmed a large region of RFA effect as visualized on MRI. Post-gadolinium imaging (Gd-SPGR and Gd-FIESTA) provided the best appreciation of RFA bone
effects. From an imaging perspective, however, it remains challenging to distinguish between coagulated bone marrow and necrotized tumour within bone, even after the administration of the contrast agent.

Similar to our efforts, Proscheck et al. used metastatic nude rats (from human breast cancer cell line) to investigate efficacy of RFA in a preclinical tumour-bearing bone model. Although they demonstrated a successful RFA treatment, their average tumour area was 19 mm$^2$, with tumour diameters ranging from 2-5 mm (23). In contrast, the BCRF electrode applicator custom designed for intraosseous applications (by virtue of internal cooling, bipolarity and the active tip size) was able to ablate large zones including tumour tissue, bone and muscle (average 12.9 ± 5.5 cm$^3$ volume of effect in this rabbit femur model). The large total ablation volume was not unexpected, as the study utilized an electrode applicator designed for human vertebral bone applied to the rabbit anatomy.

The size of the RF ablation zone achieved with a single treatment in this study incorporated both bone and surrounding thigh soft tissues. Since the thigh muscles have a high thermal conduction coefficient, the extent of ablation which occurs in the soft tissue is greater than would occur within a large bone structure (27). Yet despite the size mismatch, the ablated volume of the lesions is encouraging from a translational perspective as they are relevant in application to the human metastatic spine (Note: this electrode applicator has since been clinically approved for use in the treatment of human vertebral metastases). The safety of the BCRF electrode applicator has been previously demonstrated in a porcine vertebral study in which the ablation zone generated (under an equivalent treatment protocol) was contained within the vertebral body in all cases (27).

The clinical indication for use of the BCRF probe is in the treatment of human vertebral metastases, yet this study evaluates tumour ablation within the femur. The evaluation of the probe is limited in this context as to date no consistent large animal vertebral tumour model exists. The VX2 lapine femur model was considered a reasonable alternative that allowed the evaluation of tumour involved bone tissue. Limitations of this model include the size mismatch (as described above) and the use of a direct local injection protocol for tumour development rather than the development of metastatic disease. A VX2 tumour model of the spine is described
in the rabbit, but is representative primarily as a neural model of epidural disease, whereas the femoral injection model focuses on bony disease. Finally, while structural changes were evaluated histologically, no mechanical testing was performed to evaluate the impact of BCRF treatment on bone strength. Previous work has shown no change in the bone strength of rabbit femora post RFA treatment up to two months (25).

3.5 Conclusions

Structurally large VX2 tumour volumes within bone were successfully ablated, stimulating new bone formation in the periphery of the targeted treatment zone. The ablation zone was best distinguished from remaining tumour and healthy tissues using contrast enhanced MR imaging, although histologically, viable appearing osteocytes and tumour cells were observed in some treated regions. The existence of select sporadic viable tumour cells within the RF ablation zone in a subset of animals motivates the desire for further improvements in zonal ablation.

Table 4. RF ablation volume measured on day 28 in healthy and tumour-bearing femora.

<table>
<thead>
<tr>
<th>MRI Sequence</th>
<th>Bone tissue (n=12)</th>
<th>Soft tissue (n=12)</th>
<th>Total Treatment (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPGR</td>
<td>1.92 ± 0.39</td>
<td>7.36 ± 3.15</td>
<td>9.28 ± 3.40</td>
</tr>
<tr>
<td>SPGR + Gd</td>
<td>1.80 ± 0.32</td>
<td>13.16 ± 6.12</td>
<td>14.96 ± 6.35</td>
</tr>
<tr>
<td>FIESTA</td>
<td>1.84 ± 0.48</td>
<td>10.72 ± 4.90</td>
<td>12.56 ± 4.91</td>
</tr>
<tr>
<td>FIESTA + Gd</td>
<td>1.73 ± 0.36</td>
<td>13.02 ± 5.53</td>
<td>14.75 ± 5.57</td>
</tr>
</tbody>
</table>

Table 5. Tumour volume developed in the injected femur, measured on day 14 and 28

<table>
<thead>
<tr>
<th>MRI Sequence</th>
<th>All femora (n=12), day 14</th>
<th>Treatment group (n=6), day 28</th>
<th>Sham group (n=6), day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPGR</td>
<td>1.36 ± 0.47</td>
<td>0.30 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>SPGR + Gd</td>
<td>1.46 ± 0.53</td>
<td>0.24 ± 0.16</td>
<td>2.56 ± 0.68</td>
</tr>
<tr>
<td>FIESTA</td>
<td>1.43 ± 0.56</td>
<td>0.43 ± 0.36</td>
<td></td>
</tr>
</tbody>
</table>
**Table 6.** Summary of histological parameters based on TRAP stained femora

<table>
<thead>
<tr>
<th></th>
<th>Tumour treated (n=6)</th>
<th>Tumour Sham (n=6)</th>
<th>Healthy treated (n=6)</th>
<th>Healthy Sham (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Oc</td>
<td>6.9 ± 6.7</td>
<td>99.2 ± 100.6</td>
<td>1.6 ± 1.8</td>
<td>5.8 ± 6.2</td>
</tr>
<tr>
<td>Large Oc</td>
<td>7.2 ± 5.8</td>
<td>63.3 ± 25.6</td>
<td>1 ± 1.3</td>
<td>4.8 ± 4.4</td>
</tr>
<tr>
<td>Small Oc</td>
<td>6.7 ± 9.2</td>
<td>135 ± 65.6</td>
<td>2.2 ± 2.3</td>
<td>6.7 ± 6.1</td>
</tr>
</tbody>
</table>

**Figure 13.** Flow chart of the experimental design.
Figure 14. BCRF electrode applicator placement procedure. The fluoroscopic x-ray shows the BCRF electrode within a tumour-bearing femur. The straight arrow points to the radiopaque band used to define the lesion location and the curved arrow points to the secondary temperature probe outside of the bone.
**Figure 15.** AE1/AE3 staining and MR images of tumour-bearing femora. (a) and (b) Tumour growth on day 14, (c) Tumour growth on day 28 (without treatment). (d) demonstrates the VX2 tumour cells which partially occupy the bone on day 14 (a), and almost completely occupy the bone on day 28 (c). (d) Treated tumour-bearing femur showing the RFA region of effect (\(\bullet\)) that has ablated the tumour within RF treatment zone. (e) High magnification representation of untreated VX2 cells and (f) high magnification of a treated VX2 zone. (g) and (h) MR images of the femora highlighting untreated and RF treated tumour respectively.
Figure 16. MRI sequences of a healthy rabbit femur pre and post RFA treatment, demonstrating distinct treatment effects in the marrow and soft tissue post ablation. (a) FIESTA pre-treatment, (b) SPGR pre-treatment, (c) FIESTA+Gd post treatment, and (d) SPGR+Gd post treatment.

Figure 17. Newly formed bone (green outline) in a healthy treated femur. (a) low magnification H&E stain with enhanced staining in the treatment region, new bone at the edges of the treatment region (arrow) and the electrode applicator track (filled arrow). (b) corresponding high magnification view of the new trabecular-like bone and the abundant osteoblasts (short arrows).
Figure 18. TRAP staining, demonstrating osteoclasts activity in (a) Tumour (T)-bearing untreated sham control, (b) Treated Tumour (TT)-bearing femur, (c) Healthy sham (with healthy bone marrow, BM), and (d) Treated healthy femur (with RF treated bone marrow, TBM). Osteoclasts are notably increased in the untreated tumour group.

Figure 19. High magnification image of osteocytes in a tumour-bearing RFA treated femur. (a) empty lacunae, (b) stained apoptotic lacunae and (c) unstained intact osteocytes in the treated region of the cortex. (d) osteocytes from the untreated region of the same femur.
3.6 References


3.7 Appendix: Generation of a pre-clinical model to evaluate a novel internally cooled probe design for RFA bone tumour ablation

Prior to conduction of the study presented in chapters 2 and 3, we completed a series of preliminary experimentations to develop an appropriate animal model and refine the RF probe and treatment parameters in collaboration with Baylis Medical Company (Industry-Matched Grant, Ontario Centres of Excellence). A brief summary is presented here.

**Aim 1:** To develop a small animal disease model appropriate for the initial tests of bone-targeted RFA devices.

**Methods:**

To characterize the time-course of bone tumour development, an orthotopic VX2 rabbit carcinoma model was utilized. Prior work by the senior investigators as well as published literature by others suggested that epidural spinal metastasis can be modeled using VX2 cells, but challenges remain in modeling bone specific vertebral metastases. We developed and characterized a femoral-specific diseased pre-clinical model recognizing clinical relevance in that weight bearing long bones of the extremities (i.e. femur), as well as the vertebral spine, are the most common and important sites of bone metastases. We determined the appropriate cell injection technique and the time point following injection after which bone lesions will be treated with RFA.

10 New Zealand White (NZW) rabbits were utilized and inoculated with VX2 cells in the femur. Cell injection numbers were varied, as well as cell injection techniques (lateral cortical drill hole versus intercondylar distal femoral injection). The time-course of bone tumour development was tracked following injection through MR imaging.

**Key Findings:**

- Determined the optimal cell suspension volume (200 µl), injection technique (intercondylar), and time following injection to subsequently treat bone tumours (two weeks) with RFA (Figure 20).
• Refined MR imaging parameters (through trial and error using various sequences and MRI coils) to achieve results that best highlighted tumour features and treatment effects in intramedullary disease. These parameters were used in subsequent projects (presented in chapter 3) studying RFA treatment effects.

Figure 20. Left: original tumour model development technique explored in our preliminary studies. We utilized larger cell volumes, a lateral cortical injection route, and a three week inoculation period. This technique was abandoned since we experienced femoral fracture in the specimens. Right: The final injection technique included lesser tumour volume and an intercondylar approach with inoculation time of two weeks which appeared promising and was used in the study presented in chapter 3.

Aim 2: To improve the design and operation of a novel internally cooled bone-targeted RF probe in the developed pre-clinical model.

Methods: 16 NZW rabbits were used. 200 μl of VX2 cell suspension (~5x10³/μl) was injected, with RFA treatment at three weeks following injection. We studied varied RFA treatment parameters (65 to 80°C probe tip set temperature, 10-30°C/min ramp rate, 11-15 min treatment duration, 30-50 W maximum power). Performance was judged by animal intra-operative clinical responses (e.g. heart rate as a surrogate to pain experience under anaesthesia, the heart rhythm, pulse pressure, capillary refill time, mucous membrane colour and respiratory rate, depth, and rhythm), and stability of the RF generator unit to complete the procedure without system errors or abruptions.
Evaluation of ablation regions (size and shape) was also conducted in phantoms and \textit{ex vivo} in rabbit derived soft tissue (muscle) as evaluated by use of a heat sensitive camera.

**Key Findings:**

- Recognized limitations with use of grounding pad (including incidence of the skin burn at the ground pads, and live residual tumour (Figure 21)) leading to change in design from a monopolar to a bipolar cooled delivery system (OsteoCool®, Baylis Medical Company, Mississauga, ON).

The bipolar design enabled shortening of the active probe tip length (from 3 to 2 cm) while maintaining the ability to create large lesion volumes (>5 cm$^3$). [Note: The probe was designed to yield desired ablation volumes for clinical application to metastatic vertebrae, as the spine is the most common and prevalent site of metastases]. This refined probe design is referred to in thesis as the BCRF (Bipolar Cooled Radiofrequency) probe (OsteoCool®, Baylis Medical Company, Mississauga, ON). The BCRF probe was found to create ellipsoid treatment volumes in rabbit tissue (of approximately 4x2 cm in soft tissue and 3x1.5 cm in the femur bone) (Figure 22 and Figure 23).

- Settings determined for use in the rabbit femur were: 65 °C probe tip set temperature, 10 °C/min ramp rate, 50 W maximum power and 15 minute treatment duration.
Figure 21. Sections of cooled RFA treated VX2 tumour within the femur. (a) dissected, decalcified and fixed femur bone sectioned in half, demonstrating the treated tumour (TT) as well as treated marrow (TM) regions distal and proximal to the tumour. (b) MR image of the same cooled RFA treated femur showing regions of necrotic and treated tumour cells (measuring approximately 2 cm in length). (c) H&E histology section of the same femur, showing treatment effect spanning approximately two thirds of the femur. The centre oval is the VX2 tumour which has been treated completely throughout and at the distal edges. However, the proximal edges demonstrate live tumour cells in conjunction with necrotic cells. (d) magnified H&E section demonstrating treated tumour (TT) and live tumour (LT). These primary results from the original cooled RFA electrode were significantly improved upon through the bipolar cooled design (BCRF electrode).
Figure 22. Representative BCRF lesion generated in rabbit VX2 soft tissue tumour demonstrating the untreated tumour (a), and the ablated tumour (b) corresponding to the gross dissection of the sample (c).

Figure 23. Lesion formation in the rabbit thigh muscle using the bipolar cooled design captured with an infrared camera.
Chapter 4
Helical coil electrode radiofrequency ablation designed for application in osteolytic vertebral tumours – initial evaluation in a porcine model

Background: Radiofrequency ablation (RFA) is emerging as a complementary treatment for vertebral metastases. Traditional RFA induces frictional heating leading to tissue necrosis in a local minimally invasive manner. Limitations of conventional probes, particularly in osseous tissues, include small and incomplete zones of ablation that leave residual viable tumour. To address this, we have developed a new RFA coil electrode design for bony applications. The design uses a higher frequency (27.12MHz vs. the current 450-600Hz) and a coil geometry that enables coupling of a magnetic and an electrical field to generate larger and more comprehensive treatment zones.

Purpose: The purpose of this work was to evaluate the feasibility and safety of this RFA design, Bone Coil electrode, in vertebral bodies.

Methods: Refinement of the RFA Bone Coil electrode design and heating protocol was initially carried out in gel phantoms, muscle tissue and ex vivo pilot vertebrae. Under institutional approval six healthy Yorkshire pigs (40-50 kg) received a sham and an RF treatment respectively in two adjacent cervical levels (note: no readily available in vivo preclinical tumour model of a suitable scale exists for device testing). While the Bone Coil is designed for minimally invasive deployment in human vertebrae, a surgical approach was required due to the high bone density in healthy porcine vertebrae and to facilitate the placement of thermal sensors. The ventral cervical spinal region was exposed and a coring drill bit was used with continuous saline irrigation to create a cylindrical path into the vertebral body for RFA electrode placement. The Bone Coil electrodes, placed within an introducer, were inserted in the drilled cylindrical pathways. The electronic circuit was completed by four grounding pads applied to the dorsal skin of each animal. Probe positioning was confirmed by fluoroscopy. Treatment was delivered for 10 min at 20 W (n=1), 25 W (n=1) and 30 W (n=4). To monitor the thermal rise and for safety, two fiberoptic probes recorded temperatures in the centre of each coil and near the spinal foramen of the tested level. Post procedure, animals were neurologically monitored for two weeks. MR imaging (with and without Gadolinium contrast agent) was completed immediately post treatment and at
14 days. The MR images were segmented to evaluate the ablation volume (AmiraDEV5.2). H&E and TUNEL histology were used to evaluate the effects microscopically.

**Results:** Comprehensive treatment of the porcine vertebrae was accomplished as demonstrated by the temperature monitoring, MR imaging post treatment and histology. Large zones of ablation (treatment volume: $3.72 \pm 0.73 \text{ cm}^3$, sham volume: $1.98 \pm 0.16 \text{ cm}^3$, p<0.05) were obtained, confined within the vertebral body. Maximum recorded temperatures in the treated levels ranged from 66.1 to 102.9°C, with no significant temperature rise outside of the vertebrae (38.2 ± 1.5 °C). Ablation effects on MR images were best visualized at day 14. Histology revealed comprehensive homogeneous coagulative necrosis with little peripheral signs of repair. Mobility, neurological responses and behaviour post-procedure and at two weeks were normal and consistent with pre-procedural examination.

**Conclusions:** This study established the ability of a bone-targeted RFA coil design to perform in an osseous environment, creating large ablation volumes with no neurologic sequelae. Thermal ablation effects of the RFA treatment were clearly distinguished from the much smaller mechanical and thermal effects seen in the sham vertebrae (due to core drilling) and corresponded to the homogenous necrosis visible on histology.

*Manuscript has been submitted to The Spine Journal.*

Pezeshki PS, Davidson SRH, Akens MK, Murphy K, McCann C, Sherar M, Whyne CM, Yee AJ.

**Co-authorship Statement**

Padina Pezeshki: Grant writing and funding acquisition, study design, probe design and development, treatment protocol development, surgical assist, MR Imaging acquisition, segmentation and analysis, histology sample preparation and analysis, neurological examination, statistical analysis, writing of manuscript.

Sean Davidson: Grant writing and funding acquisition, probe design and development, treatment protocol development, data acquisition, manuscript editing.

Margarete Akens: Surgical assist, manuscript editing.

Kieran Murphy: Grant writing and funding acquisition, manuscript editing.
Claire McCann: Soft tissue RFA Coil design and development

Michael Sherar: Grant writing and funding acquisition, supervision, manuscript editing.

Cari Whyne: Grant writing and funding acquisition, study design, supervision, manuscript editing.

Albert Yee: Grant writing and funding acquisition, study design, surgeries, supervision, manuscript editing.
4.1 Introduction

Bone metastases frequently occur in patients with advanced cancer, which presents treatment challenges that may require a multidisciplinary strategy (1). In the skeleton cancer spreads most commonly to the spine (2, 3) with metastases representing more than 90% of the tumours of the vertebrae (4). When left untreated, bone metastases can lead to skeletal related events (SRE) including pain, hypercalcemia and pathologic fractures (2, 5, 6). The use of radiofrequency ablation (RFA) in the management of vertebral metastases is increasing (7-12). In patients with painful metastasis in whom other methods have been inadequate or contraindicated, minimally invasive RFA provides an attractive alternative (13). The clinical goal of RFA in vertebral metastases is primarily pain reduction as well as tumour shrinkage prior to stabilization (1, 10, 14). Conventional radiofrequency ablation electrodes, generally developed and optimized for use in soft tissues, are integrated into a straight needle. RF devices with retractable curved electrodes (multi-tined electrodes) in an umbrella-shape geometry have also been developed in an attempt to create larger lesions than those generated using a monopolar straight needle. These devices operate in a frequency range of 450-600 kHz (15). At these frequencies, the electric field generated by the devices is localized to the immediate vicinity of the device (16). This will in turn excite the nearby ions within the tissue, causing ionic movement, heat generation and conduction. Conventional ablation electrodes therefore depend on heat conduction within the target tissue, which is reliant on the thermal properties of the tissue (17). Bone has a low thermal conduction coefficient (18). As such, comprehensive ablation of a sizable tumour in bone without charring and carbonization remains a clinical challenge (19). The other limitation of conventional RFA devices is their susceptibility to local blood flow, which carries heat away from the treatment site and limits the size of coagulation in highly vascularised tissues.

Recently, a new RFA device design has been developed, which incorporates a helical coil geometry and operates at a frequency of 27.12 MHz (20). The combination of the geometry and frequency in this design allow for induction of magnetic and electric fields inside the coil. Unlike conventional RFA technology that operates in the kHz range (which heavily relies on thermal conduction), the helical coil electrode design exploits both magnetic and electric fields leading to a uniform power deposition inside the coil, irrespective of the tissue thermal conduction properties. As a result, a larger, more uniform and comprehensive ablation zone may be produced which has the potential to address more extensive metastatic lesions. The performance
of this technology has been demonstrated in phantoms and liver tissues (20-22) and is currently being evaluated in a Phase I clinical trial for treatment of kidney and liver tumours. To apply this technology to bone it was necessary to redesign the helical RF coil geometry to enable its use within the metastatic spine. The electrode coil and introducer were modified in terms of size and stiffness to enable deployment and ablation within bone tissue.

This work demonstrates the safety and efficacy of this novel Bone Coil ablation electrode in an \textit{in vivo} porcine vertebral model. The results of the Bone Coil ablation are compared with a conventional needle electrode designed for use in bone (23). It was hypothesized that the electromagnetic fields produced by the novel ablation device design can be used to safely create larger lesions in a bony environment than conventional RFA technology.

\subsection*{4.2 Materials and Methods}

\textit{Experimental design}

Institutional approval was obtained for all animal procedures. A healthy vertebral pig model was used due to its similarity to the human vertebrae in terms of size and geometry. Furthermore, a healthy pig model was used as there is no readily available \textit{in vivo} preclinical vertebral tumour model of a suitable scale for device testing. Six Yorkshire pigs (40-50 kg) received both a sham (n=6, RF ablation electrode placement only, generator not turned on) and a radiofrequency treatment (n=6) respectively in two adjacent cervical levels (e.g. C4 and C5) accessed surgically. The outcome parameters included: (1) temperature in the centre of ablation zone and adjacent to spinal foramen, (2) neurologic status of the animals, (3) dimensions of the treatment zone (based on MR imaging and histologic analysis of the excised vertebrae) and (4) histologic evidence of ablation. Zones of ablation were compared to the results from a previous study using a similar healthy porcine vertebral model and a bone-targeted needle ablation electrode (23).

\textit{RF Ablation Device}

Prior RF coil electrode development for soft tissue tumour ablation (20) guided the current novel coil design for osseous applications. Refinement of the ablation electrode design and heating protocol was carried out in gel phantoms, muscle tissue and \textit{ex vivo} pilot vertebrae prior to the current study. A stronger coil was required for deployment in bone compared with that used for soft tissue. The bone coil was fabricated from Nitinol memory alloy (NDC, Fremont, CA),
which, due to its material properties, can assume a pre-set helical geometry following deployment through a straight cannula. The final design had the following parameters: Nitinol wire diameter: 1.3 mm, coil diameter: 9 mm, pitch: 2 mm, coil length: 8 mm and 3¾ turns. Pilot experimentations revealed that these parameters were important in achieving the mechanical strength required for deployment within human bone tissue. The Bone Coil electrode was driven at 27.12 MHz by an RF power generator (CESAR® 273 RF GENERATOR, Advanced Energy, Fort Collins, CO) and a matching network (Variomatch 1500W Digital Matching Network, Advanced Energy, Fort Collins, CO) through a flexible coaxial cable attached to the proximal end of the ablation electrode. The return path to the generator was provided by a set of four return electrodes (REM PolyHesive II; Valleylab, Boulder, CO) coupled in parallel.

**Anesthesia**

Each pig received an intra-muscular injection of atropine (0.04 mg/kg) and ketamine (15 mg/kg) prior to MR imaging and treatment. A 22G catheter was placed in an ear vein for intra-venous access. Each animal was intubated and anaesthesia was maintained by ventilation with isoflurane in oxygen (1.5-2.5%; 2L). For pain relief, prior to and after the procedure, all pigs received an intra-muscular injection of 0.05 mg/kg (0.3 mg/ml concentration) Buprenorphine (Temgesic) (Schering-Plough, NJ, US) or 0.1 mg/kg (1.5 mg/ml concentration) Meloxicam (Metacam) (Boehringer Ingelheim, CT, US) twice a day, for three days.

**Treatment procedure**

The RF bone coil is designed for a minimally invasive transpedicular insertion into a human vertebra. A surgical approach was required in the porcine model, because the density of healthy porcine vertebrae is much higher than normal human vertebrae and deployment of the coil was not possible using a minimally invasive approach. Anaesthetized animals were positioned in dorsal recumbency and a sterile surgical field was prepared, exposing the cervical region of the spine. A left-sided ventro-lateral incision was made on the neck skin, along the medial edge of the sterno-cleido-mastoid muscle. The muscles and adipose layers were then carefully separated and cut using a pair of Metzenbaum surgical scissors and cautery. The trachea, and esophagus were retracted medially, and the carotid vessels protected laterally. When the cervical vertebrae (usually C3-5) were exposed, bone wax was applied across any bleeding bone edges to minimize bleeding. A 10 mm D coring drill bit was used under continuous saline irrigation to create a
cylindrical path into the vertebral body for RF coil electrode placement. A small (~ 2 mm D) hole was also drilled in the centre of the cylindrical path for placement of a temperature sensor (Figure 24a). Bone Coil ablation electrodes placed within an insulated introducer were inserted into both cylindrical pathways (Figure 24b). The electronic circuit was completed by four grounding pads applied to the medial and lateral aspects of the animal's trunk's dorsal skin. The electrode positions were confirmed by fluoroscopy (Figure 24c). One of the two coil electrodes was connected to the generator and matching network while the other coil – the sham – remained unconnected. Treatment was delivered for 10 min at 20 W (n=1), 25 W (n=1) and 30 W (n=4). To monitor the temperature, three fiber-optic sensors (Luxtron, LumaSense Technologies, Santa Clara, CA) were located in the centre of each coil and also adjacent to the spinal foramen (Figure 24b).

**Neurologic examination**

The animals were monitored for two weeks post procedure to ensure that no iatrogenic injury had resulted. For verification and as a measure of safety, a neurological assessment (adapted from Straw et al. (24)) was used to test the animals' motor and sensation responsiveness to stimuli both prior to and post procedure (24).

**MR imaging**

MR imaging (with and without Gadolinium contrast agent) was completed immediately post treatment (day 0) and at day 14. MR imaging was performed in a 3.0T GE Signa scanner (GE Healthcare, Milwaukee, WI), using a clinical GE cardiac coil. Images were obtained in all 3 planes (sagittal, coronal and transverse) using a 256 x 256 matrix, 16 cm field of view, 3 mm slice thickness, and 0 spacing gap. T1-weighted, TR/TE:557/16.4, and T2-weighted, TR/TE:5513/125.4 (25) scans were performed for each animal. Gd-DTPA contrast agent (0.1 mmol/kg) was injected intravenously and post contrast T1-weighted images were acquired. The MR images were manually segmented to determine the ablation volume based on the hypo and hyperintensive demarcating signals that were used to identify the ablation borders (AmiraDEV 5.2, Visage Imaging GmbH, Germany).
**Histology**

Fourteen days after treatment (after the 2\textsuperscript{nd} MRI) the pigs were anesthetized and sacrificed (sodium pentobarbital, Euthanyl® 120 mg/kg). The experimental vertebrae (sham and treated) were harvested and fixed in 10\% buffered formalin. After fixation, specimens were decalcified using an EDTA protocol (26). Decalcified axial sections of the vertebrae stained with Hematoxylin and eosin (H&E) were assessed to characterize the ablation zone by measuring the area of cell death and qualitatively describing tissue changes. The effect on bone cells and the marrow cavity within and beyond the ablation zone were examined, as well as changes to the nearby neural and vertebral tissues. TUNEL staining was used to identify apoptotic cells in bone. Cell death was determined by counting the number of cells containing nuclei with apoptotic morphology that were also stained dark brown with 3,3'-Diaminobenzidine (DAB). A positive pixel count algorithm was used to quantify the ablation area. Specifically, osteocytes were evaluated for viability as judged by the presence of intact nuclei at the marginal and central zones of ablation. Bone marrow was examined for any thermally induced changes when compared to controls. The spinal cord and surrounding connective tissue were qualitatively compared with the sham control levels to provide comparative evaluation of cell morphologies and to differentiate the effects of RF ablation from those due to drilling.

**Analysis**

A paired Student's t-test was used to compare areas and volumes of effect (i.e. cell kill and morphologic changes), measured on histology and MR imaging respectively, between treated and sham groups. The volumes of ablation were also compared with those obtained with the BCRF ablation probe (OsteoCool®) as described in Pezeshki et al. (23).

4.3 Results

**Ablation volume measurements**

Large volumes of ablation were visible on both MR images and the histologic sections of the treated levels (Figure 25). Treatment extended to the intervertebral discs. T2-weighted and contrast-enhanced T1-weighted images (Figure 26) provided the best visualization of the treatment effect. A noticeable region of effect, attributed to the thermal and mechanical damage from drilling, was also observed on all sham control images and histology (Figure 25). Volumes
of ablation, determined from MR imaging, were, however, significantly larger in the treated group (3.72 ± 0.73 cm³) than the region of thermal effect (from drilling) in the control group (1.98 ± 0.16 cm³, p=0.000). Furthermore, the ablation volumes were significantly larger in the present study than those created previously in the healthy pig model with a bone-targeted straight needle ablation electrode operated at 460 KHz (2.24 ± 0.90 cm³, p=0.008) (23).

Feasibility and safety

The treatment procedure was technically successful with no system failures or challenges. The surgical access, electrode placement and ablation produced no iatrogenic injury. All animals tolerated the procedure well and were able to move immediately after the procedure. Their mobility and neurological responses post-procedure and at two weeks were normal (no sign of post-procedural lameness in the two week follow up period) and consistent with pre-procedural examination. The animals appeared to have no difficulty in normal daily activities, such as eating and playing with toys. Radiographic data confirmed no bone fracture or other defects aside from the cylindrical paths generated for therapeutic access. Similarly, imaging and histologic evaluation verified that the spinal cord and nearby soft tissues were intact and undamaged in all samples. Fibrous tissue was observed during gross dissection at the drilling site in 11 of the 12 excised vertebrae. The one cervical level without any scar tissue formation at the drill site had experienced hotter temperatures, boiling and charring during treatment. Maximum recorded temperatures in the treated tissues ranged from 66.1 to 102.9 °C, while no significant temperature rise was observed outside of the treated vertebrae (38.2 ± 1.5°C).

MR Imaging

Vertebral treatment effects were best visualized two weeks post procedure, although immediately post ablation, treatment effects were visible (particularly in the samples that received the highest power level (30W)). In the low-power levels (20 and 25W), it was more difficult to distinguish between the control level and the treated level on MRI. However, in the histology samples, the RF ablation effects were distinctly different from the effects of drilling alone at all power levels evaluated in the present study. In contrast the high-power levels were easily visible on all MR images and the imaging corresponded closely to the increased histologic damage.
In the axial view, lesions were generally triangular in shape and bounded by the cartilage growth plates that are present in the vertebrae of growing pigs. Extension beyond the cartilage plane was seen in only one sample, where higher temperatures, charring and boiling were also noted during the procedure.

The drilled pathway (a physical indentation in the trabecular architecture) was distinctly noticeable in all images as a hyperintensive demarcation in T2 and T1-weighted images. In the treated samples, a thinner hypointensive rim was observed when compared to the straight needle, low frequency ablation electrode reported by Pezeshki et al.(23). In the images of the control samples, the drilled pathway showed a much more hyperintensive signal when compared to the ablation rim of the treated samples. These hyperintensive areas were representative of the regeneration and fibroblastic composition that corresponded to our histological observation.

**Histology**

Histology confirmed that in comparison to a straight, low frequency ablation electrode, the Bone Coil electrode created larger ablation zones with more extensive necrosis and fewer signs of repair or fibrous tissue formation. A more uniform necrotic pattern of ablation was observed for these samples as compared to zonal coagulative necrosis created by low frequency ablations. Axial areas for regions of effect were measured for treated and control samples (223.20 ± 45.21 mm² and 92.99 ± 42.88 mm² respectively), and found to be significantly different using both H&E and TUNEL stained samples (p<0.001).

At low magnifications, H&E was not particularly useful in determining the ablation boundaries while the drill marks were visible. In contrast, TUNEL staining clearly revealed zones of effect on both control and treated samples that corresponded well with the features in the MR images, namely the drill paths and the ablation perimeter. In general, within the ablation zone, TUNEL staining was very intensive, including all the marrow and the osteocytes. In the H&E sections of the ablated region, all cells appeared ghost-like, with nuclear fragmentation, dark pyknotic nuclei or smudged chromatin, indicating cell death. Sporadic fibroblastic nodules were also visible in the centre of ablation. RF ablation effects were most pronounced in the vicinity around the coil probe in the central vertebral body region bounded by the cartilaginous growth plates (Figure 27 b and c, and Figure 28) At the periphery of the ablation zone just beyond the growth plates, however, staining of the cells (chondrocytes of the cartilage plates and osteocytes immediately
outside of the plates) was less pigmented and only visible in high magnification TUNEL analysis. In the outermost region, consistent with imaging, a small rim containing fibrous tissue and an active repair process was observed in both H&E and TUNEL stained sections. At high magnifications, H&E demonstrated a large zone of ablation bounded by loose fibrous tissue formation and abundant new vascularisation at the periphery and outside of the cartilage growth plate. At the edges of the ablation zone there were osteoclasts adjacent to non-viable bone, while new bone was observed being formed by osteoblasts (Figure 27 and Figure 28). In agreement with dissection observations, granulation tissue was seen at the sites of the drill and the electrode entry in all samples.

4.4 Discussion

Safe and successful ablation of vertebral tissue was demonstrated using the high frequency helical coil ablation electrode. This RFA electrode repeatedly produced large, well-confined coagulation within the cervical porcine vertebrae with an average volume of $3.72 \pm 0.73 \text{ cm}^3$. Histologically the ablation regions demonstrated comprehensive necrosis without damage to any of the soft tissues or nearby critical structures. Temperature measurement outside of the ablation zone and near the vertebral foramen confirmed a physiologically normal temperature of $38.2 \pm 1.5^\circ\text{C}$ in all procedures. While in this study we did not directly measure the spinal cord temperature, our extensive ex vivo studies prior to these pre-clinical experiments established sufficient temperature difference between the spinal cord and the ablation zone within the vertebrae. No neurologic complications were experienced by the animals and the patency of the spinal cord was confirmed by both MRI and histology.

With the increasing popularity and success of RFA procedures, efforts are geared towards creating tissue-specific devices that can address the current therapeutic shortcomings of inadequate lesion formation, charring, skin burns or nerve injuries (27-29). Ablation using traditional RF relies predominantly on thermal conduction in the target tissue. Traditional RFA electrodes used in bones have been designed for and tested in soft tissues and therefore fall short in creating comprehensive lesions in osseous tissue with relatively low thermal (0.15-0.3 W/m-\text{oC}) and electrical (0.01 s/cm) conductivities (18). Application of a helical coil geometry and a higher frequency range (27.12 MHz vs. the 450-600 KHz conventional range found in straight or multi-tined ablation electrodes) enables an ablation procedure that is not as dependent on the
thermal properties of the bone tissue. The geometry/frequency combination utilized resulted in both a circumferential electric field and an axial time-dependent varying electric field (directed along the axis of the coil) and is essentially uniform in the coil interior. The ensuing axial currents and the eddy currents produce uniform power deposition leading to homogenous necrosis necessary for comprehensive tumour ablation. The histologic results demonstrate this highly uniform necrosis in the bone with few signs of repair within the ablation zone at the two week period. In contrast, histologic results of conventional RFA, demonstrate a more zonal pattern of necrosis, which is most necrotic in the hottest zone adjacent to the ablation electrode and trails off as the distance increases from the ablation electrode tip (23, 30). It has been shown in the literature that residual or sporadic live tumour cells have remained in or near RF ablated regions. These live cells may have migrated to the centre of ablation from the peripheral residual tumour or may have developed post-procedure (31-33). Since the Bone Coil electrode offers an electromagnetic power deposition that leads to uniform heat generation and a comprehensive ablation (as demonstrated by our TUNEL stains), it may reduce the potential for residual tumour seeding or spread post-RFA.

In this model, the ablation extended to the intervertebral discs because the height of the cervical porcine vertebrae was short relative to the ablation electrode diameter. In order to take advantage of an electromagnetic field in this solenoid geometry, design of an ablation electrode with fewer turns (and therefore shorter than the current size) would likely not produce enough coagulation (an ideal geometry would be a long coil with tightly wound turns (20)). As such, the coil ablation electrode design was large for the size of the porcine cervical vertebrae in the anterior/superior direction. As a surgical ventral approach was required, the experiment was focused in the cervical spine. In contrast, the OsteoCool® BCRF straight electrode (23) was placed percutaneously in the lumbar porcine spine. Since the comparison was made between the total volumes of ablation created by each electrode, the variance in the vertebral levels should not affect the comparison.

We also observed that the zone of ablation using the coil probe appeared mostly contained in the central vertebral body bounded by the porcine vertebral growth plates (Figure 27 b and c). Interestingly, histologically some RF effects were observed in a small perimeter on the side of the growth plate furthest away from the coil (Figure 28). This is likely due to the insulative
effects of the cartilaginous growth plates which partially contained the thermal propagation and
RF effects to within the central vertebral body.

Given that conduction plays an important role in conventional radiofrequency treatment, any
ablation electrode placed in a well-perfused soft tissue and away from a major heat sink (such as
a large vessel or spinal fluid) will create a larger ablation zone than it would in an osseous tissue.
Consequently, placement of the Bone Coil electrode in a soft tissue lesion is expected to create
larger ablation volumes simply due to the thermal conduction properties in comparison with the
high density healthy porcine bone. It is encouraging to see the ablation zones achievable in this
bone model. This shows promise when considering potential application of high frequency RFA
to diseased vertebrae.

Vocal and esophageal tissue complications have been reported in the literature with
ventral/anterior cervical procedures (34). In our study, all animals demonstrated normal signs of
curiosity and appetite without any complications related to the surgical or ablation procedure.
Despite the drilling procedure, followed by RFA heating, we observed no signs of fracture or
bone weakening grossly, radiologically or histologically. This finding was consistent with
findings of Martel et al., Yamamoto et al. and Cantwell et al. who examined bone fractures post
RFA in dogs, rabbits and porcine long bones respectively (with an exception of two humeral
fractures in pigs, likely attributed to drilling) (30, 35, 36). Clinically, post-RFA fractures have
been reported (37)(27), however, it has been suggested that these fractures may be the result of
instrumentation and holes drilled into the body which largely weaken the bone, as opposed to the
effects of RFA heating on the bone tissue (35).

Consistent with previous work examining RFA effects using MR imaging, we were able to
successfully identify the treatment zone in all samples. It was possible to distinguish between
the treatment effects and the thermal drilling effects in the sham levels. All of the imaging
details corresponded well with histologic findings. Similar to other published works, we found
best visibility with T2-weighted and contrast-enhanced T1-weighted images demonstrating the
best demarcation of ablation boundaries seen on the day 14 images (23, 25, 38).

Although the coil geometry and frequency range make this design robust and powerful in
generating a uniform ablation zone, its use is limited to osteolytic, osteoporotic or osteopenic
bones where the memory alloy coil shape can be easily deployed and unwound into the osseous
structure. In patients with osteolytic tumours this ablation electrode will be placed through a conventional trochar and vertebral cannulation in a minimally invasively manner (the feasibility of coil deployment was previously determined in cadavers during preliminary testing). Whereas conventional straight needle electrode placement through a transpedicular approach allows creation of a lesion only in the lateral portion of the vertebrae, (unless a lateral para-pedicular approach is utilized), transpedicular placement of this coil electrode can yield a lesion positioned centrally within the vertebrae as illustrated in Figure 6.

A limitation to the present coil design is the potential challenges with deployment in osteosclerotic bone lesions. In such cases a bone-targeted conventional needle geometry (23) that can be directly inserted remains a viable option. Other limitations in the present study include evaluation in healthy rather than diseased tissues, although having a sham control level within each animal was helpful to distinguish the effect of RFA from those of coil placement.

4.5 Conclusions

This study demonstrated the safety and potential for efficacy of a high frequency helical coil-shaped RFA electrode that exploits complex magnetic and electric fields to create large well-confined ablation zones in healthy porcine vertebrae. The large zone of effect was easily visualized with standard clinical MR imaging and confirmed through histologic analysis. Procedural safety was verified based on imaging, histology, neurologic evaluation and thermal measurements. Further preclinical and clinical trials are necessary to demonstrate successful application of the device for its intended use in the tumour-bearing skeleton.
Figure 24. Images from the *in vivo* experiments. (a) After exposing the cervical vertebrae via an anterior approach, cylindrical paths (white arrows) were drilled into two adjacent levels (one sham control level, one treated level) with a central hole drilled in the middle of the cylindrical paths to place temperature sensors. (b) RFA Bone Coils were inserted into the cylindrical paths and insulated introducers covered the exposed part of the electrode wires. Fiber optic thermal probes were placed into the holes in the centre of the ablation zone as well by adjacent spinal foramen. (c) Fluoroscopic lateral x-rays confirmed RF electrode placement.

Figure 25. (a) Axial, (b) sagittal and (c) coronal T1-weighted contrast-enhanced MR images demonstrating treated (at 30W) (green) and sham (purple) levels two weeks post treatment.
**Figure 26.** Axial MR images of a treated level on day 14 demonstrating the ablation region. The two parallel central lines in the images correspond to the drilled pathway created for the Bone Coil electrode. (a) Contrast enhanced T1-weighted shows a large hypointense ablation zone, (b) T2-weighted shows slightly hyperintense ablation zone (c) T1-weighted shows a less obvious hyperintense ablation zone.

**Figure 27.** Images of representative levels from the porcine vertebrae demonstrating the region of thermal effect; Top row: sham control (result of drilling and RF electrode placement without any power applied through the electrode). Bottom row: RFA treated (at 30 W), (a) Axial T2-weighted (b) TUNEL stained section (c) H&E stained section.
Figure 28. High magnification images of a representative RFA treated level (at 30 W) from the porcine vertebrae demonstrating the outer rim of the ablation region (a) TUNEL demonstrating staining of a few osteocytes (thin arrow), distinct from the highly stained inner ablation zone (b) H&E staining demonstrating fibrous tissue formation (thick arrow), empty lacunae of several osteocytes (thin arrow), new blood vessels (curved arrow) and areas of rapidly forming bone (curly arrow).
Figure 29. Transpedicular placement of the high frequency coil geometry electrode (left) versus the conventional frequency/geometry RFA electrode (right). Due to the uniform electromagnetic power deposition within the coil, a more consistent heating is expected with this device. In contrast, needle ablation electrodes rely on thermal conduction, which create a zonal heating pattern with the hottest areas closest to the electrode tip.
4.6 References


4.7 Appendix: Development and *ex vivo* testing of a new RF Bone Coil system for osseous applications

Prior to conduction of the study presented in chapter 4, we designed and developed the probe and conducted *ex vivo* validation through three preliminary projects summarized below.

**RFA treatment using the Bone Coil Probe system:**

**Objectives:** To develop a probe for deployment and RFA delivery in bone and to characterize a new RF Bone Coil probe in *ex vivo* treatment within gel phantoms, muscle and bone.

**Aim 1:** To ensure successful RF Bone Coil probe deployment in vertebral bone.

**Methods:** Utilized pig lumbar vertebrae (45-60 kg, *n*=6) and elderly human cadaveric vertebrae (*n*=5) to test deployment with the initial RF Bone Coil Probe design. Various aspects of the design were modified to improve robustness in device deployment. Outcome measures included successful deployment (visualized via fluoroscopy) and physical ability in retracting the Bone Coil probe intact.

**Key findings:**

- Repeated attempts in deploying the coil into healthy pig or younger human vertebrae failed, and deployment in a few osteopenic cadaveric samples was challenging, leading to the recognition that there may only be a select population of vertebral tumours (i.e. osteolytic vs. osteoblastic) that may be appropriate for the specific use of the RF Bone Coil probe design (Figure 30). Conceptually, the Bone Coil probe possesses the potential for a greater regional tumour ablation zone, upon successful deployment, when compared to a single needle probe design.

- A stiffer and stronger coil than initially planned was required for deployment in bone. This was achieved by reinforcing the coil design with an increased Nitinol wire (1.3 mm) and decreased coil diameter (final parameters: D 13 mm, pitch 2 mm, H 8 mm and 3¾ turns) (Figure 31).

- Earlier attempts had included incorporation of the Bone Coil into the commercially available Kiva® vertebroplasty system (Benvenue Medical, Santa Clara, CA). However
the new stiffened coil could no longer be deployed using their system. As such we
returned to an in house deployment system which was further modified and enhanced to
allow deployment in the bone. Modifications included use of a stiffer cannula and a side
channel to control deployment direction.

- The increased probe stiffness led to bending of the introducer which was resolved with
two modifications: (1) the 14G steel tube (originally glued to the handle) was machine
threaded such that the tube is now screwed into the handle, and (2) a larger tube (11G)
was placed over the exposed portion of the 14G tube to strengthen the design against
bending.

- Other modifications to the probe explored with the design team included: coil diameter,
annealing parameters, heat shrink placement and material, applicator design, and
attachment to the applicator. These improved deployment and the robustness of the
design.

- The new design was successfully tested in senile human vertebrae (n=2). A
transpedicular approach was used to deploy the final design coils under fluoroscopy into
two levels of the cadaveric spine of an elderly osteopenic woman.
Figure 30. Preliminary results demonstrating Bone Coil deployment (a) successfully in an elderly human vertebra (including a temperature probe inserted in the centre), and (b) unsuccessfully in a healthy dense porcine vertebra.

Figure 31. Final Bone Coil and introducer design for human vertebrae.

**Aim 2:** To demonstrate the effectiveness of the RF Bone Coil probe in creating large consistent ablation zones in gel phantoms and soft tissue and bones.

**Methods:** Utilized gel phantoms, soft tissue (muscle), *ex vivo* porcine and cadaveric vertebrae to evaluate ablation and lesion generation using the RF Bone Coil. Outcome measures comprised temperature of the treated tissues, heat leakage and size of the treatment zone.
Key findings:

- Consistent thermal lesions were generated in gel phantoms, soft tissues and soft tissue-filled vertebrae. These lesions were made using the final design for human vertebrae and a modified smaller design (with a 90º bend) for the *in vivo* porcine experiments of chapter 4 (Figure 32 and Figure 33).

- Determined RF Bone Coil treatment parameters (10 minute treatment of 3.5 min at 50 W, followed by 6.5 min at 35 W) based upon thermal coagulation effects observed in the phantoms and muscle. This knowledge guided the tumour simulation experiments (*ex vivo* Aim 3, and Chapter 5).

**Figure 32.** Progressive lesion generation in a gel phantom using the Bone Coil modified design for *in vivo* porcine experiments of chapter 4. Large ablation volumes were achieved.
Figure 33. Representative lesions created using the Bone Coil probe in *ex vivo* soft tissue, demonstrating large zones of ablation with uniform coagulation.

**Aim 3:** To determine a treatment protocol that can effectively ablate bone tissue using *ex vivo* bone samples.

**Methods:** Individual porcine (n=5) and human cadaveric vertebrae (n=8) filled with soft tissue (pork ground meat) as a model for metastatic involvement were used. Ablation treatment was conducted using the RF Bone Coil Probe with a number of different protocols. Outcome measures included temperature measurement within the coil and adjacent to the spinal canal and visual evaluation of the ablation zone post treatment visible following vertebral sectioning (Figure 34 and Figure 35).

**Key Findings:**

- Ablation of the dense porcine trabecular bone tissue was achieved that corresponded well with the size of the coil (Figure 36).

- Comprehensive ablation of the soft tissue (i.e. simulated tumour) inside the vertebrae was achieved.

- Spinal cord temperatures measured were higher than desired but were explained by the *ex vivo* nature of the testing and lack of perfusion. Further *in vivo* tests (Chapter 4) confirmed the safety of ablation using the Bone Coil system (Figure 37).

- Developed an ablation protocol for *in vivo* testing to gain maximal lesion with minimal spinal cord heating and procedure time.
Treatment protocol was adjusted based on temperature feedback as compared to the impedance feedback mechanism of the soft tissue coil protocol (which can more commonly result in charring).

**Figure 34.** Porcine vertebral preparation for preliminary RF ablation experiments. (a) harvested healthy lumbar vertebra, (b) drilling into the anterolateral wall using a 11 mm diamond core to create a path for the coil, (c) RF Bone Coil probe insertion into the cored path.

**Figure 35.** *Ex vivo* experimental set up for validation of Bone Coil RF ablation in bone. (a) Fiber optic temperature sensors placed in the centre of the coil and the spinal canal, (b) an x-ray of the vertebra housing the RF Bone Coil electrode, (c) specimen in water with grounding pads, the Bone Coil and the temperature sensors in place.
Figure 36. Treated healthy porcine sample. (a) Bone core removed to visually inspect the pathway and the canal. (b) treated bone core (left) demonstrates the effects of ablation as compared to the untreated bone core (right).

Figure 37. Sample temperature rise during heating of a vertebra.
Chapter 5
The impact of bone-targeted Radiofrequency Ablation (RFA) alone and in combination with Percutaneous Vertebroplasty (PVP) on tumour involved vertebral strength and mechanical stability

**Background:** Radiofrequency ablation (RFA) and percutaneous vertebroplasty (PVP) are used independently and in combination to treat metastatically involved vertebrae with the aim of relieving pain, reducing tumour burden and providing bony mechanical stabilization. However the effects of RFA on vertebral strength and mechanical stability, alone and followed by PVP, are unknown.

**Purpose:** The purpose of this work was to characterize the ability of RFA alone and in combination with PVP to improve strength and mechanical stability in tumour involved vertebrae.

**Materials and Methods:** Human cadaveric motion segments (n=12) were used to simulate metastasis by artificially creating a cavity filled with fresh human tumour samples. Tumour tissue was treated with two bone-targeted RFA devices (BCRF and Bone Coil electrodes), followed by PVP. Under axial loading, spinal canal narrowing was measured in the intact specimen, after tumour simulation, post-RFA treatment and following cement hardening. Samples were CT-imaged post-treatment and finally loaded to failure. We assessed tumour shrinkage, cement volume and the pattern of cement distribution within the vertebra, spinal canal narrowing under load as a measure of vertebral mechanical stability, and ultimate failure load representing vertebral strength. We compared our results with previously published data on PVP treated and PVP+Laser Induced Thermotherapy (LITT)-treated vertebrae.

**Results:** The presence of tumour led to an increase in spinal canal narrowing under load. While RFA treatment of the tumour led to volume shrinkage and cavitation, it further increased canal narrowing. Post-PVP, the samples in which cement distribution extended to support the posterior aspect of the vertebral body (BCRF group), yielded stability of the posterior vertebral body wall equivalent to intact (non-tumour involved) vertebrae. However, in the Bone Coil treated group, while there was more comprehensive tumour ablation, cement distribution was
limited to the anterior and lateral vertebral body due to the narrow helical path created by the probe with limited restabilization. No significant difference was found in ultimate load to failure comparing the BCRF+PVP and Bone Coil+PVP treated vertebrae.

**Conclusions:** RFA and PVP alone lead to increased canal wall narrowing under load, but in combination, they can significantly reduce posterior wall stability if sufficient tumour shrinkage and cavitation is coupled with a pattern of cement deposition which extends to posterior vertebral body.

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Co-authorship Statement

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5.1 Introduction

The spine is one of the most common sites of metastatic involvement, which can lead to pain, pathologic fracture and neurologic complications, significantly impacting quality of life for cancer patients (1-4). Clinically, in treating spinal metastases we aim to reduce tumour burden and the resulting pain (1), improve vertebral mechanical stability, and reduce the risk of complications related to pathologic fracture and the potential for subsequent neurologic impairment (5). Radiofrequency ablation (RFA) and percutaneous vertebroplasty (PVP) are two modalities currently used in the treatment of spinal metastases to relieve pain, ablate tumour tissue and mechanically stabilize vertebrae (5-8).

The clinical definition of spinal stability includes mechanical stability as well as absence of any neurologic damage, deformity or pain(9). The ability of the spinal column to maintain its pattern of displacement under physiological loading defines spinal stability from a mechanical perspective (9). The mechanical stability of the vertebral body is represented in its resistance to deformation in both the axial and radial directions under load, which includes movement of the posterior vertebral body wall, and contributes to ultimate strength. Our cadaveric work focuses on understanding vertebral strength and movement of the posterior vertebral body wall as an important variable influencing mechanical stability.

RFA is a minimally invasive tumour ablation procedure with increasing popularity for application in the spine. Traditionally, RFA involves placement of an ablation electrode in the target tissue, application of grounding pads to the skin and delivery of alternating current (AC) through the circuit. The AC excites ions near the ablation electrode leading to resistive heating and coagulative necrosis of the target tissue or tumour. In this manner, RFA depends on the conductivity of the target tissue and free travel of the electrons through the circuit. As such, RFA has been limited in its ability to create large ablation zones and is associated with iatrogenic injury (such as skin burns at the site of grounding pads or nerve injuries near the electrode placement) (10-12). In the spine, these limitations are further augmented due to the lower thermal and electrical conductivities of the osseous tissue and the presence of adjacent critical neurologic structures (12). While most RFA electrodes have been developed for soft tissue applications, recent developments in RFA technology have sought to address these limitations by
designing probes optimized for use in an osseous environment (13)(Chapter 4). Two new RFA electrodes have been developed specifically for the treatment of vertebral metastases: a bipolar cooled radiofrequency ablation electrode (BCRF, OsteoCool®, Baylis Medical Company, Mississauga, ON) and a helical coil ablation electrode (Bone Coil, University Health Network and Sunnybrook Research Institute, Toronto, ON). Both devices have shown preclinical safety in porcine models and represent potential advances in RFA osseous technology (13)(Chapter 4).

PVP involves the injection of bone cement (i.e. polymethylmethacrylate (PMMA)) into osteolytic, osteoporotic or fractured vertebrae in order to restore mechanical stability and reduce mechanical pain. Generally, 2-5 ml of cement is injected percutaneously through a 10-14 G cannula into a vertebral body. Potential complications of PVP include: cement leakage, cement or tumour embolization, radiculopathy, thermal damage to the bone and surrounding tissue, systemic toxicity due to monomer circulation and local exothermic reactions (14-16). In the metastatic spine, post-PVP cement leakage has been reported in up to 85.7% of all procedures with 10% reporting leakage into the spinal canal (17). Although cement extravasation is common, clinical concern is focused on the less common, however serious, neurologic complications that can arise from extravasation into the spinal canal. The high rate of extravasation may be explained by breaches in the vertebral cortex or increased intravertebral pressures generated during the procedure in the presence of tumour (17, 18). Further, improvements in vertebral mechanical stability have been demonstrated when PVP is applied following thermal tumour ablation, with volumetric reductions in the tumour tissue allowing for improved cement fill (19). The ability to safely, easily and effectively create volumetric reductions in tumour tissue within an osseous environment may be beneficial to PVP success.

Clinically, in the metastatic spine, RFA is increasingly being used as a part of a combination therapy with PVP. This combined RFA+PVP approach has demonstrated reduced pain and improved mobility in patients (7, 20-22). Theoretically, it has been suggested that performing RFA prior to PVP may be beneficial as tumour shrinkage achievable with RFA will create additional space in the vertebrae, enabling cement flow in areas of osteolysis which require stabilization (19, 23). PVP will also displace tumour tissue when cement is injected. It is therefore preferable that displaced or embolic tumour be ablated and non-viable. We hypothesize that the application of RFA designed for the osseous environment will improve the efficacy of
PVP in the treatment of vertebrae with osteolytic metastases in a simulated cadaveric model. Specifically, the objective of this study was to quantify the ability of RFA treatments designed for the osseous environment (using BCRF and Bone-Coil System devices) to reduce tumour volume leading to improved cement fill patterns, and superior post PVP mechanical strength and stability in a cadaveric vertebral tumour model.

5.2 Materials and Methods

Study Design

Six human cadaveric spines (T12-L5) were obtained and CT-imaged (Toshiba Aquilion ONE, Tokyo, Japan) to rule out any pathological fractures (4 males, 2 females, age: range 62-96 years, mean 78 ± 12.5 years). The spines were sectioned into three-level motion segments (L5-L4-L3 or L2-L1-T12, n=12) and cleaned of any excess soft tissue (24). From each spine, the two specimens were randomly assigned to one of the BCRF or Bone Coil treatment groups, ensuring an equal number of upper and lower segments were assigned to each treatment group (18). Each motion segment was tested under axial loading at four stages: intact, post metastasis simulation, post RFA treatment, and following PVP treatment as per the protocol of Ahn et al. (19). Load induced spinal canal narrowing (LICN) of the middle vertebra was measured at each stage (19, 25). After treatment, CT imaging was repeated to visualize tumour shrinkage and the pattern of cement distribution. Finally, all specimens were loaded to failure (Figure 38). A repeated measures ANOVA was used to compare LICN of each testing stage within groups. A one-way ANOVA was used to compare mechanical stability between the Bone Coil and BCRF groups with previously published data from our laboratory which similarly evaluated PVP alone and PVP with laser induced thermotherapy (LITT) in vertebrae with simulated metastases (19) (PASW software, Version 18.0, Chicago, IL, USA). Final axial loading to failure was used to compare vertebral strength post combined RFA+PVP treatment. In order to compare the outcomes from the two RFA electrodes, the ultimate load to failure from the two spinal motion segments obtained from each spine were assumed to behave as paired samples for statistical comparison (18).
**Initial preparation**

Specimens were frozen and stored at -20 °C. Prior to testing each specimen was thawed overnight at 4 °C and brought to physiologic temperature of 37 °C for testing in a digital saline bath (Isotemp 205, Thermo Fisher Scientific Inc., Waltham, MA, USA). In order to secure the motion segments into the testing apparatus, and access the spinal canal, the posterior elements of the superior and inferior vertebrae of each motion segment were removed leaving the middle level intact. Measurements of the middle level were made using calipers in order to make an estimated volume calculation of the vertebral body.

**Tumour simulation**

As per Ahn et al. (19), in order to artificially simulate an osteolytic lesion a cavity was created in the centre of the L1 or L4 vertebra, using a coring drill bit (16mm diameter). Fresh human tumour (equivalent to 12% of the calculated vertebral body volume; high grade carcinoma procured from the uterus, ovary or omentum) was placed in the centre of the cavity. A bony end cap was made out of the cored bone piece and used to seal the cavity with bone cement.

**Radiofrequency Ablation**

BCRF ablation system: The bipolar cooled radiofrequency probe (17G, 1.518 mm outer diameter) was designed for use in the metastatic spine. It takes advantage of an internal cooling mechanism together with a bipolar circuitry (without the requirement for an external grounding pad) to improve lesion generation in an osseous environment. The radiofrequency generator (Kimberly-Clark Pain Management RF Generator, Mississauga, On, Canada) delivers electrical energy at a frequency of 460 KHz, with a maximum hardware output power of 50 W. The system uses a default controlled ramp rate of 10 °C/min with a typical ablation duration of 12 minutes.

Bone Coil ablation system: The Bone Coil system uses a non-conventional radiofrequency to create large and more consistent lesions by taking advantage of improved power deposition in the tissue, independent of tissue connectivity or heat sink limitations. This probe is made out of a Nitinol memory alloy (NDC, Fremont, CA) in the shape of a coil (wire diameter 1.3 mm, coil diameter 13 mm, pitch 2 mm, height 8 mm, 3⅓ turns). The Bone Coil electrode is driven at
27.12 MHz using an RF power generator ((CESAR® 273 RF GENERATOR, Advanced Energy, Fort Collins, CO) and an impedance matching network (Variomatch 1500W Digital Matching Network, Advanced Energy, Fort Collins, CO) through a flexible coaxial cable attached to the proximal end of the ablation electrode. The return path to the generator is provided by a set of three grounding pads (REM PolyHesive II; Valleylab, Boulder, CO) that were secured inside the walls of a phantom box filled with water. A typical ablation duration for this device is 10 minutes.

Treatment: A transpedicular approach was used for the initial cannulation (11 G, Osteosite Bone Biopsy Needle, Cook, Canada), through which BCRF electrode placement, bone coil deployment and PVP cement injection occurred. The cannula and electrode positions were confirmed using fluoroscopy (6). RFA was completed using each ablation electrode as per its respective protocol. For the BCRF electrode, 12 min of ablation was conducted at a maximum electrode tip temperature of 70 °C at 460 KHz. To keep the body moist and enable the conduction of resistive heating, a saline injection was delivered during the treatment (<2 ml) through a secondary bone biopsy needle in the contralateral pedicle. At instances where the system stalled due to dry vertebral conditions, multiple treatments were conducted to achieve a total of 12 min of ablation. For the Bone Coil electrode, a monopolar transpedicular approach was used. The coil was deployed using an in house polymer guide handle with an extending 20 cm-long cannula which was reinforced by an insulated introducer. An opening at the tip of the introducer, aligned with the fin on the polymer guide handle indicated the orientation of the coil deployment. The Bone Coil is unwound inside the tissue orthogonal to the introducer. A 10 min ablation protocol was used inclusive of an initial 3 min @ 50 W followed by 6.5 min @ 35 W power delivery at 27.12 MHz (Figure 39).

**Percutaneous Vertebroplasty**

PVP was completed on each motion segment using a transpedicular approach. As per the manufacturer's protocol, radio-opaque PMMA cement (Surgical Simplex P, Howmedica Osteonics Corp., Mahwah, NJ, USA) was prepared, by mixing a 20ml to 40g liquid monomer to powder ratio). Approximately 3-4 ml of cement was injected into each specimen depending on
the size of the vertebra. Injection was stopped when resistance was felt or extravasation from the cannula was observed.

**Mechanical testing and outcome measures**

All specimens were tested under axial loading at a rate of 1600 N/s to 800 N on a servo hydraulic materials testing machine (MTS Bionix 858; Eden, MN, USA) at four stages: intact, post metastasis simulation, post RFA treatment, and following PVP treatment. A curved brass canal displacement gauge connecting the posterior arch and the midpoint of the central posterior vertebral body wall of each spinal motion segment was used to measure the spinal canal narrowing under axial loading (Figure 40). The measurement of load induced canal narrowing (LICN) is a non-destructive test that measures the bulge of the posterior vertebral body wall into the canal under axial compressive loading. Larger LICN values correspond to greater instability in the posterior wall and an inclination to fracture. LICN results from the RFA and combined RFA + PVP treatments were compared to PVP alone and PVP + LITT treatments as reported by Ahn et al. (19).

At the completion of the non-destructive testing, all samples were CT imaged to monitor tumour shrinkage and the pattern of cement distribution (Figure 41). Samples were then loaded under axial compression to failure. Post testing, the samples were axially sectioned and the evidence of tumour ablation and patterns of cement distribution evaluated by visual examination (Figure 42).

### 5.3 Results

**Tumour simulation results**

A total of 12 cadaveric samples were tested, however data from gauge measurements of two samples were discarded due to strain gauge malfunctions (BCRF: n=4 and Bone Coil: n=6). As expected, introduction of the tumour in each motion segment caused an increase in load induced spinal canal narrowing (LICN) compared to the intact samples in both groups (intact: 0.043 ± 0.038 mm, tumour: 0.102 ± 0.063 mm, p=0.003, n=10).
**RFA results**

Despite irrigation, it was technically more challenging to proceed with the ablation using the conventional-frequency-based bone-targeted BCRF electrode (at 460 KHz). The system failed on several occasions despite saline infusion requiring multiple ablations to complete a full treatment protocol. [Note: this is a feature of the system that increases safety of the device in a clinical setting.] In contrast, the Bone Coil, which relies on higher power deposition as opposed to tissue conductive properties, easily and successfully completed the ablation in every case. Probe positioning and electrode deployment was straightforward except in one sample where the helical coil tip of the electrode punctured through the anterior wall of the vertebrae.

Tumour ablation was achieved using both electrodes with more comprehensive coagulative necrosis evident in the Bone Coil group. Similarly, tumour shrinkage (based on volume determined from the post treatment CT images) was greater using the Bone Coil electrode (residual tumour volume post ablation: BCRF = 2.43 ± 0.75 mm³, Bone Coil= 1.42 ± 0.32 mm³, p=0.058). Additionally, the cavity created post RFA treatment had a pattern unique to each electrode. The helical coil electrode led to creation of a pathway following the coil geometry, whereas a straight path was seen in the BCRF treated samples. Post-ablation LICN was increased in both groups, leading to less stability of the posterior vertebral body wall (tumour: 0.10 ± 0.06 mm, post-RFA: 0.22 ± 0.20 mm, p=0.03, n=10) (Figure 43).

**PVP results**

Cement extravasation from the anterior vertebral wall was observed only in the sample that had been punctured with the Bone Coil electrode. Leakage from the posterior vertebral wall at the location of the strain gauge mounting was also observed in one of the 12 samples. The volumes of injected cement were quantified based on the post treatment CT images. There was no significant difference in the cement volume injected between the two groups (2.89 ± 1.77 and 3.74 ± 1.77 ml for Bone Coil and BCRF electrodes respectively, p=0.22). LICN was significantly improved post-PVP in all samples treated with BCRF (BCRF: 0.126 ± 0.057 mm, BCRF+PVP: 0.04 ± 0.04 mm, p=0.02). In the Bone Coil group, there was a high variation in the results with LICN improving only in half of the samples (Bone Coil: 0.32 ± 0.25 mm, Bone
Coil+PVP: 0.25 ± 0.21 mm, p=0.54) (Figure 43). In between group comparisons, a trend was found demonstrating BCRF+PVP to be more stable than the Bone Coil+PVP (205%, p=0.09).

In the BCRF group the cement was distributed along the posterior portion of the vertebral body leading to the observed reduced LICN. In contrast, in the Bone Coil treated samples the cement was mainly localized in the anterior/lateral parts of the vertebral body (Figure 41). Load to failure values were not significantly different between the BCRF and Bone Coil groups (1978.98 ± 1165.22 N and 2448.60 ± 1276.50 N, respectively, p=0.49, n=6 per group). All specimens failed at the middle column of the spine (26), indicative of a pathologic burst fracture.

5.4 Discussion

This study aimed to explore the role of radiofrequency ablation alone and followed by percutaneous vertebroplasty in the treatment of metastatically involved vertebrae. The clinical benefits of PVP with RFA have been described (5, 7, 27-32) with outcome measurements primarily based upon patients' pain scores or radiographic evaluation. To our knowledge, no other study has investigated the biomechanics of the metastatic spine following RFA and RFA+PVP in the treated spine.

We hypothesized that higher ablation volumes would lead to greater cement fill and improved vertebral strength and mechanical stability. The results of the present study demonstrated that it was the distribution pattern of cement rather than the volume of ablation that was the most important factor in improving posterior wall stability. Whereas tumour shrinkage was greater using the Bone Coil electrode, improved vertebral stability was achieved in the BCRF treated samples in which the cement fill pattern included the posterior aspect of the vertebral body. These results were consistent with available data in the literature that report improved stability when cement is distributed in the posterior vertebral body (19). Ahn et al. investigated vertebral mechanical stability post PVP alone and in a combined laser induced thermotherapy (LITT)+PVP protocol using the same model as used in the present study. Their results demonstrated that PVP post LITT improved vertebral mechanical stability under loading due to the void created by LITT leading to improved cement fill patterns. In contrast, PVP alone did not always result in a more mechanically stable vertebra as the cement distribution may occur such that posterior vertebral wall is not supported (19). In comparing our results, a trend was found
demonstrating BCRF+PVP to be more stable than the PVP alone group from Ahn et al.’s findings (210%, p=0.07). The BCRF+PVP results were similar to LICN values obtained by Ahn et al. in LITT+PVP group (p=0.94). In contrast, the Bone Coil+PVP results were similar to Ahn et al.’s PVP alone group (p=0.97) and demonstrated a trend toward lower stability than their LITT+PVP group (214%, p=0.05) (Table 7).

As well, in our BCRF treated group, we were able to obtain vertebral stabilization equivalent to the LITT+PVP group in Ahn et al. with half of the volume of cement injected, demonstrating posterior vertebral body cement fill. This supports the concept that there is no need for large volumes of cement injections (with the risk of extravasation) to achieve stabilization post PVP and highlights again the importance of the pattern of cement fill (Table 7). From a clinical perspective, although cement fill towards the posterior region of the vertebral body may be important in lessening the risk for pathologic burst fracture, it is important to consider the existing integrity of the posterior vertebral body wall. In situations where there is advanced destruction of the posterior vertebral wall by osteolytic tumour, there is greater concern for the potential of cement extravasation into the spinal canal. As such, patient selection for PVP is important and needs to consider both clinical as well as radiographic factors.

Tumour shrinkage elicited with the Bone Coil, led to creation of a cavity following the pattern of the coil, whereas a straight cavity was created by the BCRF probe. In samples treated with Bone Coil, cement was mainly localized in the anterior/lateral parts of the vertebral body. The resistance in the coiled pathway (calculated to be 7.8 times more resistive to the cement flow as compared to a straight pathway created by BCRF) may have prevented comprehensive fill of the RFA-created void. Despite the increased tumour shrinkage, this cement distribution pattern led to variable results with some specimens demonstrating increased pressure on the posterior wall under loading resulting in greater LICN. These results were consistent with the PVP-only (no ablation) group in the Ahn et al. study. Hence, the pathway created by tumour ablation (which drives cement distribution patterns) is critically important in order to achieve improved mechanical stabilization of the posterior vertebral body wall post-PVP.

Load to failure analysis provides an assessment of the overall strength of the spinal motion segment. The lack of significant difference in load to failure data in this study is similar to the
findings of Ahn et al. and may represent the high variance between samples. Consistent with previous studies that report no relation between vertebral strength and cement volume injection beyond 2 ml (33), our injection of 3-4 ml resulted in similar strengths in both groups.

Anselmetti et al. have reported improved results using a Poly-ether-ether-ketone (PEEK) implant (Kiva®) assisted vertebroplasty, where the stacked coil shape of the implant restores the height of the vertebra and serves to prevent cement leakage (34). Other studies have also reported on benefits of the PEEK implant (Kiva®) assisted vertebroplasty system (35, 36). In the osteolytic spine, combining Bone Coil RFA treatment with PEEK implant Kiva® assisted vertebroplasty may be an attractive consideration as the tumour ablation is maximized using this RFA coil electrode and the cement distribution pattern is dictated by the PEEK implant. Additionally, in patients where the posterior wall may be compromised, injection of cement into the confined space of the PEEK implant may be more reassuring.

Although previous work has demonstrated efficacy of the BCRF probe under in vivo conditions (13), there were technical challenges in completing the ablation procedure with this device in ex vivo cadaveric vertebrae. The BCRF ablation system relies on heat propagation through the ionic constituents of the cells. Therefore, in the absence of blood and perfusion in our ex vivo model, the BCRF system reached the set tip temperature quickly and was unable to conduct a full ablation protocol. As this system benefits from internal cooling and a bipolar circuitry, it may still perform better than most other conventional designs in ex vivo conditions with reduced hydration. In our experimentation, we assisted the BCRF system by providing saline irrigation through a bi-pedicular approach and/or multiple ablations in 70% of the samples. Despite this, incomplete ablation was observed upon gross examination of the samples in 2/3 of the treated levels. This observation further confirms the dependence of conventional RFA modalities on tissue properties (including thermal conduction, vascularisation, heat sink issues, blood flow and perfusion) and explains the limited ablation volumes achieved (11, 37). In contrast, the Bone Coil electrode relies on a magnetic and an electric field generated due to the coil geometry and the frequency range of 27.12 MHz. This results in higher power deposition and consistent generation of a comprehensive and homogenous ablation zone without any technical interruptions and less dependence on tissue properties or hydration (Chapter 4).
The results of our study are relevant as RFA and RFA+PVP combined modalities are common in treatment of spinal metastases. It is important to consider how these therapies affect the pattern of cement fill and its resultant effect on mechanical vertebral stability. While RFA alone can help reduce tumour pain and its local disease progression, the RFA treated metastatically involved vertebrae were found to be mechanically less stable than before treatment. This destabilization resulted from shrinkage of the tumour; additional void space created in the vertebral body led to increased canal narrowing under load. To restore mechanical stability, vertebroplasty can help by filling some or all of the created void space in the vertebra with cement. If the cement is distributed posteriorly (as in the case of BCRF+PVP and Ahn et al.’s LITT+PVP treated samples (19)), posterior wall stability can be restored to levels found in healthy vertebrae without tumour involvement. A bi-pedicular filling approach, as opposed to a unipedicular filling, may also play a role in achieving effective distribution patterns with improved stability motivating future studies.

San Millán Ruíz et al. reported on necrotizing effects of PMMA cement when injected in primary or secondary tumours of bone. They found that PMMA injection led to a rim of necrosis both macroscopically and microscopically 6 months post-implantation, likely due to the exothermic effects of polymerization (38). Through improvements in electrode design and RFA technology, bone-targeted RFA demonstrates promise in the ablation of larger volumes of vertebral tumours. The RFA+PVP combination therapy may achieve more comprehensive tumour necrosis, while allowing for more effective cement fill patterns and mechanical restoration of the spine.

A limitation of this study is that tumour simulation may not fully represent osteolytic metastases, however, it has been previously used in the literature as a metastatic model to study pathologic strength and stability (Whyne papers). In the performance of the BCRF device, the lack of perfusion hindered its ability to effectively ablate tissue. As such, ablation volumes generated using this probe ex vivo may not reflect the cavitary defects created in vivo and the resultant cement pathways. Future work to evaluate vertebral strength requires a reduction in specimen variability with respect to level, size and bone density or the consideration of these variables in a larger multifactorial analysis.
5.5 Conclusions

This study demonstrated the effect of RFA and combined RFA+PVP procedures on the strength and mechanical stability of vertebrae with simulated osteolytic lesions. While RFA is effective in tumour management and necrosis, alone it creates a cavity in the vertebral body which can reduce mechanical stability of the posterior vertebral body wall, leading to bulging and the potential for an increased risk of burst fracture initiation under axial loading. Performing PVP after RFA can improve vertebral mechanical stability, particularly if the cement can be directed to yield a distribution that supports the posterior vertebral body wall. RFA probe design and instrumentation that can ablate tumour and direct a pathway for cement fill, may provide an optimized treatment for osteolytic metastatic involvement in the spine.

Table 7. The percent change in Load Induced Canal Narrowing (LICN) values at different stages obtained in our study of metastatic tumour simulation treated with RFA+PVP, compared with those of Ahn et al. (19) using LITT+PVP and PVP alone.

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<th>Intact</th>
<th>Tumour</th>
<th>BCRF+PVP</th>
<th>Bone Coil+PVP</th>
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<tr>
<td>40.85%</td>
<td>100%</td>
<td>38.72%</td>
<td>243.95%</td>
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<tr>
<td>Ahn et al. Intact</td>
<td>50%</td>
<td>100%</td>
<td>29.7%</td>
<td>248.5%</td>
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<td>Ahn et al. Tumour</td>
<td>LITT+PVP</td>
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Figure 38. Experimental flow chart
Figure 39. BCRF (left) and Bone Coil (right) RFA electrodes

Figure 40. Schematic of the experimental simulated metastatic vertebral level. The intact vertebra was initially drilled to create a cavity in the centre of the body. Fresh tumour was then inserted to fill the vertebra and a bony cap sealed the gap. A brass metal strip instrumented with two strain gauges, was secured in the spinal column to measure load induced canal narrowing (LICN). Probe placement for RFA and PVP treatment was transpedicular.
Figure 41. CT images of cement distribution patterns on the experimental lumbar vertebrae post PVP and (a) RFA with the Bone Coil electrode, (b) RFA with the BCRF probe. Insets (top left) show x-ray images of probe placements during the procedure.

Figure 42. Axial cross sections of treated specimens: (a) tumour-bearing vertebra treated with the Bone Coil + PVP demonstrating comprehensive treatment and vertebroplasty cement (full arrow) distributed mainly anterolaterally and within tumour, (b) tumour-bearing vertebra treated with BCRF + PVP demonstrating an incomplete ablation zone (hollow arrow) and posterior cement (full arrow) under the described ex vivo experimental set up.
Figure 43. Percent change in load induced canal narrowing (LICN). Post-RFA all samples experienced increased posterior wall movement and instability due to tumour shrinkage and the resultant empty space created in the body (first two columns). More successful ablation with greater tumour shrinkage resulted in a larger change in LICN (Bone Coil-RFA). PVP post RFA significantly improved vertebral mechanical stability in the BCRF group (BCRF+PVP).
5.6 References


Chapter 6
General Discussion

6.1 Discussion and Conclusions

In this work we have improved and evaluated two bone-targeted RF electrodes and their treatment protocols for application in skeletal metastases. Each unique electrode has proven successful in the initial pre-clinical experiments without any concerns for safety or technical challenges.

The BCRF electrode, designed and manufactured by Baylis Medical Company, and refined through our collaborative efforts showed safety and efficacy in our studies, and is currently a commercially available product undergoing clinical evaluation in both North America and Europe. The Bone Coil electrode, designed and developed in house by our team, (University Health Network and Sunnybrook Research Institute), similarly was demonstrated to be safe and effective in creating large ablation zones within vertebrae.

This thesis work was motivated by the challenge associated with radiofrequency ablation in bone in generating comprehensive lesions in view of the size of metastases and the thermal and electrical conductive properties of bone (1). Some authors have expressed that the sclerotic characteristics of metastasis may be a contraindication to RF ablation (2) since this kind of lesion may not transmit heat in the same manner as soft tissue lesions and will not be destroyed by RF (3). Since, one of the limitations of RFA is its heavy reliance on good electrical and thermal tissue conduction (4), low values of conductivity for bone theoretically represent a challenge in ablation of osseous tissue (2). For instance, whereas thermal conduction coefficient of many soft tissues such as liver (the most widely RFA treated tissue historically (5)) is 0.5 watts/m-°C, thermal conduction coefficient of bone is 0.3 watts/m-°C. Similarly, whereas the electrical conduction coefficient of liver is 0.12 s/cm the electrical conduction coefficient of bone is 0.01 s/cm (6). Additionally, since conductive heating may be carried away by heavy fluid or blood flow, conventional RFA devices are also highly susceptible to the amount of vascularisation present in a tissue (7). For these reasons, when conventional RFA devices developed for soft tissues are used in bone, the lesions may be less predictable (potentially compromising safety)
and smaller in size. In addition to these inherent properties of bone, the anatomical location of the critical structures such as the spinal cord and nerve endings, render RFA treatment of osseous tissue complicated and require that factors such as a confined zone of ablation are addressed through device design.

Traditional RF electrodes function by depositing the static charges of the electrical field in the first few seconds of ablation and exciting the ionic tissue nearby, and rely on further growth of the lesion by the ensuing frictional heating that has been instigated by the initial energy deposition. This lesion growth can only continue until the moist cells are coagulated and dried up and cannot further stimulate nearby frictional and resistive heating. In traditional monopolar (450-600 KHz) RFA set ups, the electrons have to travel from the source (the electrode tip) to reach the grounding pad and enroute, in the immediate vicinity of the electrode, have a high enough concentration that tissue coagulation results. Original attempts in moving to bipolar apparatus for RFA in soft tissue was observed to create too short of a pathway between the two probes (depending on the final placement of the probes by the operator) for the electrons that would not allow sufficient tissue heating (8). In addition, placement of two probes (an active and a return probe) is technically more cumbersome (9). Another practical challenge was the high impedance immediate to the electrodes that would prevent lesion growth. The power dissipation between the two probes could not be controlled independently leading to nonuniform and small lesion size (10). These challenges were overcome in the BCRF design. The placement of the active and the return electrodes on the same probe, in addition to the internal cooling of the design, yields a short pathway for electrons but allows wider transmission of frictional and resistive heating by preventing charring and desiccation. This combination is ideal in application in the spine, where a large tumour may require ablation but the electrons do not pass by the nerve roots or spinal cord. More importantly, the short pathway means that in comparison to the conventional probes, there is less reliance on material conductivity of the tissue, since the travel route for completing the electronic circuit is shorter than it would be if the grounding was applied further away on the skin. The internal cooling in BCRF aids with heat removal adjacent to the electrode and prevents accumulation of high impedance that could reduce the size of the lesion. This design improvement in our preclinical studies demonstrated efficacy in healthy and diseased osseous application and spared the spinal neurologic structures.
In the Bone Coil design, we exploited a magnetic field in addition to the electrical field that has an even lesser reliance on tissue properties. By combining the geometry of a coil and employing 27.12 MHz RF energy, it is possible to deposit a uniform power inside the coil that can homogeneously coagulate tissue without dependence on conductive heating. This frequency is sufficient for magnetic induction and generation of electric fields inside the coil as well as around the conducting wire, which allow for larger, more uniform coagulation volumes (11). As with any RF system, conductive heating would still be present within the tissue and would particularly play a role in growing the lesion outside of the coil diameter. However the power deposition within the coil geometry would be the dominant necrotizing force. This enhanced ablative property was demonstrated in both our in vivo studies and the cadaveric tumour simulation studies where the Bone Coil created larger ablation zones in comparison to the 460 KHz BCRF electrode.

Consistent with clinical practice, we assessed our ablation outcomes with MR imaging and evaluated the ability of MRI to represent histologic outcomes. Dimensions of ablation measured in the MR images correlated well with histologic findings and were best visualized at later imaging time points (two weeks vs. immediately post-ablation) that allowed visualization of the necrotic events. Specifically, the hypointensive rim visible in the MRI sequences demonstrated the margins of ablation that corresponded to fibrous tissue found in histology. We also investigated traditional T1 and T2-weighted MR imaging in our spinal experiments. FIESTA (T2-based) and SPGR (T1-based) imaging sequences were also evaluated in our tumour-bearing femoral model along with the impact of gadolinium contrast enhancement. It has been reported that T1-weighted imaging is useful in detecting bone metastases and progression of the disease, but controversial in evaluating the therapeutic response in patients (12-15). We found that we were able to easily distinguish and evaluate the treatment effect in the porcine spine (16) and discern between drilling effects and RF ablation effects using the Bone Coil. MR imaging allowed visualization of the treatment zone in our diseased femoral model, yet it was difficult to specifically distinguish between coagulated tumour and coagulated bone marrow in our tumour-bearing femora even after gadolinium injection. Despite this, overall we found MRI to be an effective tool in assessing ablation effects. We concluded the most effective protocol to be T2-based imaging and gadolinium enhanced T1-based imaging at a time point two weeks post
treatment to allow for easy detection of cellular necrosis via imaging (note: studies of focal hyperthermia have demonstrated that depending on the tumour model, it may take between 5-7 days for the tissue necrosis to become evident (17)). As such, a time point of 5-7 days post-RFA may also produce similar results.

An understudied area with respect to RFA bone treatments is the impact of RFA on bone cells and tissue. Using the BCRF electrode (which may best demonstrate ablation at conventional RF energies in an osseous environment), we examined the RF effects on osteoblasts, osteoclasts, osteocytes, periosteum and endosteum (in addition to the VX2 tumour cells). It was found that ablation resulted in death and significant reduction of osteoclasts in both our healthy and osteolytic diseased models. As well, RFA resulted in necrotic staining of the periosteum and endosteum and the embedded osteoblasts and progenitor cells. However the treatment only sporadically impacted osteocytes, demonstrating patches of viable cells among ablated cells. This may be explained by the protective and insulating effects of the cortical shell and its mineral composition. However, the destructive impact of RFA on these cells may be limited by the evaluation at a two week time point post treatment which may be too early to fully capture the extent of osteocyte death. Finally, using the BCRF electrode, we found that new bone formation and osteoblastic activation was stimulated at the periphery of the ablation zone in every treated sample, with the regenerative potential preserved in the system.

In contrast, using the Bone Coil, we found comprehensive osteocyte necrosis and ablation as determined based on TUNEL staining, (in addition to significant tumour volume shrinkage in our cadaveric study), which suggests the potential for improved tumour ablation using the higher frequency device if probe deployment is successfully achieved. With the Bone Coil fewer signs of repair and regeneration were observed in the bone at the two weeks post-RFA time point, which may be due to more comprehensive coagulation achieved in the area.

Following RFA with the BCRF probe in the VX2 model, as expected, we found a significant reduction in the tumour cell volume. However, isolated live tumour cells were visible at two weeks post ablation in one third of the treated samples. Possible explanations for this may be incomplete ablation, tumour reseeding or migration of tumour cells from the peripheral untreated zones. While the ability to ablate tumour cells was demonstrated in an osteolytic model, clinical
investigations by others have suggested refraining from treating osteoblastic lesions with RFA as bone tissue does not conduct heat well. Instead, these authors have suggested utility of cryoablation, or plasma-mediated RF ablation where use of initial tissue dissolution and ionization will allow frictional heat conduction (3, 18). In this context, the mechanism of action of the Bone Coil might have potential in tumour cell ablation in osteoblastic disease, however coil deployment in sclerotic tissue limits the feasibility of this approach based on the current device.

The impact of RFA and combined RFA+PVP procedures on vertebral stability is important as they become more routine in the treatment of spinal metastases (18-21). As such, we explored the mechanical stability of these procedures using a cadaveric simulated osteolytic metastatic spinal motion segment model. While RFA can be very effective in controlling tumour burden and pain reduction (as reported by many (2, 22, 23)), we found that the shrinkage of the tumour resulting from RFA alone structurally weakens the treated level, increasing motion of the posterior vertebral body wall into the canal under axial loading. This mechanical instability is directly related to the success of the ablation procedure; that is, the more successful the ablation is in coagulating the tumour and reducing its volume, as demonstrated by our Bone Coil electrode, the more mechanically unstable the vertebra becomes. This can be intuitively explained as the tumour shrinkage creates a cavity that provides no stability under loading. In contrast, if the treatment is only somewhat successful in creating tumour necrosis (as is the case with many current systems), less space is freed up in the vertebral body and the compromise to stability may be more limited. In these studies we also observed that the BCRF electrode had difficulty completing circuit in the less hydrated cadaveric specimens, despite achieving complete ablation in vivo (highlighting important nuances in conventional frequency RFA).

Injection of bone cement to fill the RFA-created-void seems a logical approach to counteract the elevated vertebral mechanical instability. However, the distribution of the cement into the posterior portion of the vertebrae is critical in order to reduce posterior wall bulging (as an indicator of burst fracture initiation risk). Our results combined with the PVP-alone results in Ahn et al. (24), demonstrate that RFA and PVP on their own are inadequate in improving stability. In isolation both procedures led to a less stable structure than the untreated metastatic vertebrae. However, in combination, they can be effective in tumour shrinkage and improving
vertebral mechanical stability, when cement is well distributed. This combination was successful with small cement injection volumes following RFA and may limit the potential for leakage with the creation of cavitory defects for the cement fill. It was observed that the path created by the initial instrumentation is very important in the pattern of cement distribution and may allow for control and prediction of cement fill patterns. Clinically, vertebroplasty is sometimes supplemented with vertebrogram for improved visualization of vertebral and epidural vascular structures with the goal of improved cement distribution and leakage prevention. However, this does not aid in improving safety of the procedure and it may cause contrast leakage into the disc space that fails to drain and confounds the detection of cement leakage (25). Avoiding cement leakage through the creation of cavitory defects would reduce the need for such supplementation.

In conclusion, in this thesis we have developed novel bone targeted RFA probes and established their safety and efficacy in generating large volumetric lesions within bone. We have determined the effects of RFA on bone cells and tissue and confirmed effective utility of MR imaging in treatment assessment. We also demonstrated that RFA is effective in tumour shrinkage and can improve vertebral strength and mechanical stability when combined with cement injected in the posterior region of the vertebral body.

6.2 Future Directions

In order to understand the longer-term impact of RFA on both tumour and bone, longer duration pre-clinical studies are required. Our results with BCRF, similar to those of Tillotson's (26), suggested incomplete ablation of osteocytes at two weeks post treatment, whereas osteocyte necrosis was complete with the Bone Coil at this time point. Lundskog reported instantaneous necrosis of osteocytes after 30 seconds of 50 °C heat exposure next to an implant (27) and Yamamoto et al. reported osteocyte shrinkage at 7 days and complete death at 30 days (28). These variable results may be indicative of experimental and technology differences, probe placement or perfusion variability, and warrant further exploration of our novel electrodes over longer time durations.

We observed new subcortical trabecular formation at the periphery of the ablation zone in our lapine femoral model study. Other treatment strategies such as bisphosphonate and photodynamic therapy have also been shown to generate new bone post treatment (29, 30). It
would be of interest to investigate the quality of the newly formed bone post-RFA in longer duration studies.

It has been suggested that thermal therapies and heat treatments precondition tumour cells for DNA ionization through radiation therapy (31, 32), a condition that can be achieved through RFA in combination with radiation therapy. However specific parameters, dose and duration and order of treatment have not been widely investigated. Similarly, Goldberg et al. suggested improved sensitivity to doxorubicin, when the tumour was first ablated (33). As such, studies looking at combined effects of RFA and chemotherapy or radiation therapy should be designed to examine the synergistic effects of such dual modalities. If initial RFA can pre-condition and sensitize tumours to further treatments such as chemotherapy and radiation therapy, a lower dosage of each may be required, which may be helpful from a patient health reserve perspective and cost effective for the healthcare system.

While we established that RFA prior to PVP is beneficial, and hypothesized that Bone Coil probe together with the commercially available Kiva® implant augmentation system may be able to significantly reduce tumour volume and improve vertebral mechanical stability, experimental studies validating this hypothesis remain to be conducted. In addition experimentation with RFA in combination to other augmentation methods such as balloon kyphoplasty and Skyphoplasty (34) would increase our understanding of vertebral mechanical stability and confirm the effective role of RFA in such dual strategies.

Previous studies of a larger, soft tissue version of our Bone Coil electrode have examined the geometry and frequency-dependent radial and axial absorption rate density and power deposition of the coil electrode using COMSOL (35, 36). It is important that similar computer modeling studies (using COMSOL or ANSYS HFSS 15.0 module) are also conducted for our Bone Coil electrode to confirm the presence of electrical and magnetic fields at 27.12 MHz. Such studies have recently been initiated and can further help optimize other effective sizes of the coil that may be helpful for different sized individuals or sites. Furthermore, the computer models will need to be validated both using an acrylamide/bovine serum albumin gel phantom (an initial controlled ex vivo set up) as well as in bone tissue.

Clinically, it is important to investigate the efficacy of the BCRF and the Bone Coil electrodes in
a suitable patient cohort. The BCRF electrode (OsteoCool®), has both FDA and Health Canada regulatory approvals and is commercially available, thus work on clinical evaluation with this probe is at a more advanced stage. Ongoing studies currently using the BCRF probe include investigation of the short and long term efficacy of the system as an adjunct to spinal stabilization surgery or vertebral cement augmentation procedures, and examine patient's pain and quality of life improvement as primary outcome parameters.

6.3 Contributions

Contributions of the current thesis include the following:

1. Advancing new research knowledge on the effects of RFA on bone tissue, bone cells and tumour cells within bone, highlighting the differences in outcome based on the RF technology (probe design and frequency used).
2. Advancing pre-clinical and scale-up animal models for the evaluation of RF effects on bone.
3. Contributing towards the design development of two novel therapeutic RFA bone probes, one of which is currently undergoing early phase clinical study.
4. Demonstrating the ability of novel bone-targeted RFA devices to safely create large lesions within vertebrae.
5. Quantifying the ability of RFA alone and in conjunction with PVP to stabilize metastatically involved vertebrae, highlighting the importance of cement distribution patterns.
6.4 References


